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Research Article

Intravascular CO_2 Injector: An Initial *in vivo* Feasibility Study of the Prototype

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Abstract

Purpose: Iodinated contrast is the gold standard in endovascular procedures. However, adverse effects after injection of iodinated contrast, such as contrast-induced nephropathy, limit its use. Intravascular injection of medical-grade Carbondioxide (CO_2) is widely accepted as a safe alternative to iodinated contrast, but its handling and manual delivery system may pose risks to patients, such as room air contamination. To address these issues, we developed an automated, microprocessor-based system for delivery of CO_2 as a contrast agent. This study aimed to test in an animal model *in vivo* the clinical feasibility of this prototype system, comparing it with iodinated contrast injection as the reference standard.

Methods: Ten Large White pigs were randomized into two groups: iodinated contrast (n=5) and CO_2 (n=5). All animals were subjected to angioplasty of the left renal artery with either iodinated contrast or injection of CO_2 with the prototype system, according to group assignment. Laboratory blood gas analyses were performed pre, peri and postoperatively. Renal function was monitored by measuring serum creatinine preoperatively and at 48 hours postoperatively. Animals were kept under clinical observation for 48 hours after the procedure.

Results: Angioplasty was technically successful in all animals in both groups, with no need for additional imaging with iodinated contrast in the CO_2 group. There were no technical failures associated with the prototype CO_2 injector. There was no clinical or radiological evidence of contamination with room air. All laboratory data were within the normal range. Angiographic images obtained in the CO_2 group were subjectively considered of inferior quality.

Conclusion: The prototype intravascular CO_2 injector was suitable for the proposed procedures and effective in obtaining images, allowing successful procedures without unexpected complications in agreement with previous positive prototype *in vitro* results. The present results demonstrate the initial technical feasibility of the device.

Keywords: Angiography; Carbondioxide; Contrast media; Durable medical equipment; Endovascular procedures; Interventional radiology

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Introduction

Cardiovascular diseases have become in recent decades the leading cause of death by disease in the Western world driving the development of new diagnostic and treatment technologies [1]. Major advances have been achieved in the diagnosis and treatment of cardiovascular diseases with the use of Digital Subtraction Angiography (DSA), in which images are acquired while injecting a radiologic contrast agent into the blood vessels.

Iodinated contrast with a high viscosity solution is currently the most commonly used contrast agent for DSA. However, it has several side effects with accompanying risks to patients. About 1% of people are allergic to iodine, which may cause anaphylactic reactions on injection resulting in serious toxic effects and even rarely in death [2-6]. In addition, the use of iodinated contrast material may aggravate pre-existing heart failure and lead to acute renal dysfunction, known as contrast-induced nephropathy requiring dialysis in some cases [7-9].

CO_2 proved to be an efficient contrast agent over the last decades, and its intravascular use is well supported by the literature [10-24]. Studies in animal models and in humans have shown that intravascular injections of CO_2 with a volume of 100 to 200 cm^3 cause no significant changes in vital signs [25-28]. With the advent of DSA in 1980, CO_2-based angiography became a useful diagnostic tool, particularly for patients hypersensitive to iodine-based contrast media or with renal impairment [23,29]. When injected, CO_2 displaces blood creating a small bubble that moves with blood flow before being dissolved and eliminated by the blood passing through the lungs. This short time is sufficient to acquire a series of images similar to those obtained with the use of iodinated contrast [30].

There is at present, a striking disparity between the uses of these two contrast media although it has been long accepted that both agents are safe and effective in obtaining images. This difference is particularly relevant and even intriguing, considering that a significant proportion of patients with cardiovascular disease have or will have kidney disease due to a combination of several clinical factors, such as age, hypertension, diabetes, and even repeated kidney injury due to frequent use of high-osmolar iodine-based contrast media during imaging tests.

This mismatch occurs mainly because the manual preparation of CO_2 injections is time consuming and attention demanding, and the delivery systems are often adapted for the capture of CO_2 from

high-pressure, high-capacity cylinders that are commonly present in the operating room, attached to the laparoscopic device. Even recent injection systems, using a plastic reservoir bag, pose a risk of room air contamination that cannot be detected by the human eye, with potentially catastrophic consequences [31-33]. These risks and technical difficulties, coupled with the benefits of using CO_2 instead of iodinated contrast, have prompted the development of a portable, automated, microprocessor-based system for delivery of CO_2 as a contrast agent.

The current study was therefore designed to test in a porcine model *in vivo* the initial feasibility of a microprocessor-based prototype system for CO_2 delivery during DSA, named "intravascular CO_2 injector", by comparing it with injection of iodinated contrast as the reference standard.

Materials and Methods

This was a technical feasibility study using an experimental design. All procedures were conducted at the Experimental Research Center of our institution. The prototype CO_2 injector was custom-manufactured by the laboratory of a private company, following the principal investigator's instructions. A previous *in vitro* analysis of the device performance was conducted independently at the Biomedical Engineering Laboratory of an independent institution, which approved the prototype for *in vivo* use in animals. The current study was approved by the research ethics committee and animal care and use committee of the institution.

Eleven adult male large white pigs weighing 42 to 53 kg were used in the study. One animal was selected for a pilot study aimed at determining the optimal parameters of the intensity of radiation produced by the fluoroscopy unit and the optimal frequency of image acquisition while injecting CO_2. In this *in vivo* feasibility evaluation, we chose to simulate an endovascular procedure of balloon angioplasty of the left renal artery in 10 animals, which were randomized to the prototype intravascular CO_2 injector or to the intravascular iodinated contrast injector. The latter was chosen as the parameter for comparison because iodinated contrast angiography is the gold standard method for vascular imaging. All experimental procedures were performed using a mobile C-Arm digital subtraction imaging system (Philips BV 25 Gold, Philips Medical Systems, Netherlands).

The animals were randomly divided into two groups

Group 1 (n=5): Angiography of the aorta just below the diaphragm and left renal artery and renal balloon angioplasty with injection of iodinated contrast (Ioxaglate meglumine; Hexabrix®, Guerbet, Aulnay-sous-Bois, France) using an automated injector (LF Angiomat 6000, Siemens, Erlangen, Germany).

Group 2 (n=5): Angiography of the aorta just below the diaphragm and left renal artery and renal balloon angioplasty with injection of CO_2 as the contrast agent using the prototype CO_2 injector developed for this study which was operated in continuous mode for delivery of a continuous micro flow of CO_2 aiming to prevent contamination with room air (Figure 1).

The procedures in group 2 were compared with those in group 1 (considered the gold standard for the purpose of evaluation) in relation to possible complications of the methods and the ability to complete the procedure using only CO_2.

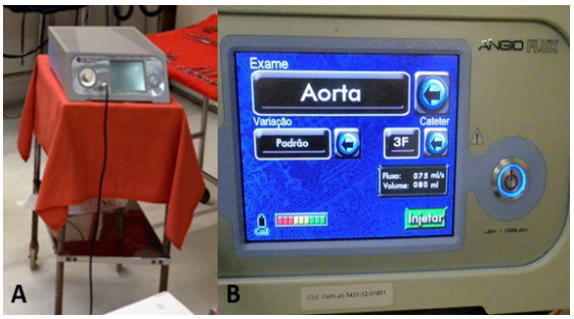

Figure 1: A) Prototype intravascular CO_2 contrast injector. B) LCD touch screen monitor of the prototype CO_2 injector.

Serial laboratory blood gas analyses were performed at three predetermined time points to monitor blood parameters for possible changes caused by CO_2 injection: 1 - At the beginning of the procedure; 2 - Immediately after the completion of angiography (with CO_2 or iodinated contrast); 3 - At the end of the procedure, immediately before removal of the arterial sheath. Arterial blood was withdrawn through the side port of the vascular introducer inserted in the femoral artery. Renal function was monitored by measuring serum creatinine. The reference values for normal serum creatinine levels in pigs are 1.0 to 2.7 mg/dL [34]. Blood samples were collected the night before and 48 hours after the procedure. During the 48-hour observation period, the animals remained under care at the laboratory, including monitoring of animal behavior and basic motor skills, with free access to water and regular pig chow.

Animals underwent daily clinical assessments throughout the study period to monitor their general health status. Only animals considered clinically healthy were used in the study. At the end of the study period, all animals were killed under general anesthesia.

Surgical technique: All anesthetic procedures were performed under the supervision of a veterinarian. The animals were preanesthetized with intramuscular ketamine (5.0 mg/kg) and midazolam (0.25 mg/kg) mixed in the same syringe. Fifteen minutes after injection, the marginal ear vein was catheterized with a 20G catheter for induction of anesthesia with 20 mg/kg of sodium thiopental. Animals were intubated with size 6 endotracheal tubes, and anesthesia was maintained with halothane. Analgesia was maintained with fentanyl, at an initial dose of 5 µg/kg, followed by continuous intravenous infusion of 0.4 µg/kg/min. During mechanical ventilation, pulse oximetry and capnometry were used for continuous evaluation of pulmonary function.

The same surgical team following standard endovascular techniques performed all procedures. Before surgery, the skin in the femoral region was shaved and cleaned with chlorhexidine gluconate 2%. Access to the right femoral aorta was established with an 18G needle y ultrasound-guided puncture, and a short 5F sheath was then implanted. After systemic heparinization with 5000 IU of sodium heparin, a 5F pigtail angiographic catheter was introduced and advanced over a hydrophilic guide wire for catheterization of the aorta, followed by aortography to locate the renal arteries. The contrast agent was then injected, according to group assignment, as follows: iodinated contrast - 10 mL, at a flow rate of 10 mL/s (Figure 2); CO_2 - 20 mL, at a flow rate of 10 mL/s (Figure 3).

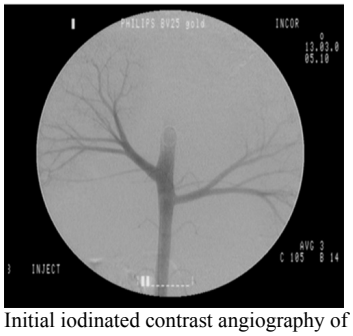

Figure 2: Initial iodinated contrast angiography of the aorta.

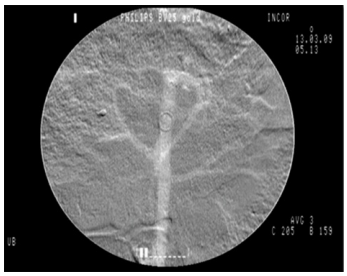

Figure 3: Initial CO_2 contrast angiography of the aorta.

Selective catheterization of the renal artery was performed using C1, JIM and/or KMP catheters, selected according to the individual anatomy of the animal, followed by renal arteriography with iodinated contrast in group 1 and CO_2 in group 2, both under apnea. Renal artery angioplasty with a 4x20 mm balloon (nominal pressure) was then performed, followed by completion arteriography with the specific contrast agent of each group at the same volume used in the initial angiography. The catheter and guide wires were removed, followed by manual compression of the puncture site for 15 minutes and compression bandaging.

Evaluation of the quality of angiographic images: Arteriograms obtained with CO_2 and iodinated contrast agents were subjectively evaluated for the quality of images produced. No quantitative score was used. Two vascular surgeons involved in the study analyzed the acquired images to determine whether they could identify the animals' renal arteries and perform renal angioplasty with technical success. A positive outcome was defined as the successful performance of angioplasty with CO_2 without the need to use additional images obtained with iodinated contrast as a complement.

Results

The renal angioplasty procedures were technically successful in 100% of cases. No procedures performed with CO_2 required additional imaging with iodinated contrast as a complement, i.e., all scheduled procedures were performed using only CO_2, achieving a positive outcome in the prototype testing.

There were no technical failures associated with the use of the prototype intravascular CO_2 injector. During the procedures using the prototype system, the device was operated stably with no clinical or laboratory evidence of contamination with room air.

The only type of complication was vasospasm of the distal renal artery, occurring in four cases (two related to the use of iodinated contrast and two related to the use of CO_2). The occurrence of these spasms was attributed to catheter manipulation of the renal artery, with spontaneous resolution in all cases as observed on the control arteriogram.

Blood pH and arterial partial pressure of carbondioxide ($PaCO_2$) values obtained pre, peri and postoperatively are shown in tables 1 and 2 respectively. In both groups, all parameters of blood gas analysis were relatively stable over time and laboratory data were within the normal range, with no difference between groups.

The greatest variation in pH was observed in animal no. 1 from the CO_2 group, which showed a decrease in pH from 7.4 (initial) to 7.17 (immediately after CO_2 injection), stabilizing at 7.39 at the end of the procedure (Table 1, Figure 4). This variation was attributed to the presence of a CO_2 concentration of 72.4 mmHg in the blood sample collected immediately after injection of CO_2 (before metabolization in the lung), which decreased to 38.3 mmHg postoperatively, a level close to the initial value of 35.1 mmHg (Table 2, Figure 5). Nevertheless, both the initial and final values were within the normal range.

n	Contrast agent	Initial	Post-contrast	Final
1	CO_2	7.40	7.17	7.39
2	Iodinated	7.27	7.25	7.28
3	CO_2	7.38	7.30	7.31
4	CO_2	7.26	7.28	7.36
5	Iodinated	7.38	7.30	7.36
6	CO_2	7.40	7.38	7.39
7	Iodinated	7.26	7.28	7.35
8	Iodinated	7.37	7.30	7.37
9	CO_2	7.46	7.40	7.42
10	Iodinated	7.40	7.36	7.38

Table 1: Blood pH values obtained in the two groups at three different points in time (arterial blood gas analysis).

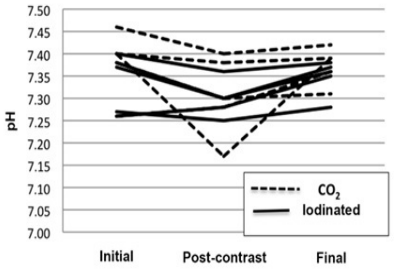

Figure 4: Graphical presentation of the 3 blood pH measures performed in each animal of the two experimental groups (arterial blood gas analysis).

All animals in both groups had normal creatinine levels with normal renal function after the procedure (Table 3). In the CO_2 group, three (out of five) animals showed a decrease in creatinine levels (animals no. 1,6 and 9), and two had increased levels (animals no. 3 and 4), but within normal limits. In the iodinated contrast group, two animals had an increase in serum creatinine levels (animals no. 5 and 7), two animals showed decreased levels (animals no. 2 and 10), and in one animal creatinine levels remained stable (animal no. 8). All these values were within the normal parameters for pigs.

n	Contrast agent	Initial	Post-contrast	Final
1	CO_2	35.1	72.4	38.3
2	Iodinated	51.4	53.2	50.4
3	CO_2	43.9	54.6	52.6
4	CO_2	57.9	58.3	45.4
5	Iodinated	46.5	52.2	49.0
6	CO_2	40.6	43.2	41.2
7	Iodinated	61.9	58.1	52.5
8	Iodinated	45.2	47.0	49.2
9	CO_2	37.0	38.9	41.6
10	Iodinated	46.7	47.5	48.8

Table 2: Blood $PaCO_2$ values (in mmHg) obtained in the two groups at three different points in time (arterial blood gas analysis).

$PaCO_2$ = arterial partial pressure of carbondioxide.

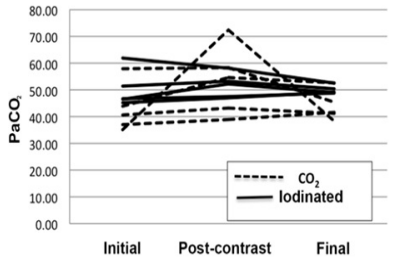

Figure 5: Graphical presentation of blood $PaCO_2$ (in mmHg) in the two experimental groups (arterial blood gas analysis).

n	Contrast agent	Preop	48 h Postop
1	CO_2	1.66	1.45
2	Iodinated	1.45	1.3
3	CO_2	1.61	1.7
4	CO_2	1.7	1.8
5	Iodinated	1.55	1.6
6	CO_2	2.6	2.3
7	Iodinated	1.5	1.8
8	Iodinated	1.7	1.7
9	CO_2	1.9	1.7
10	Iodinated	1.8	1.6

Table 3: Serum creatinine values obtained in the two groups preoperatively and postoperatively.

Reference values for normal creatinine levels in pigs: 1.0 to 2.7 mg/dL [34].

None of the animals had contrast-induced nephropathy, regardless of the type of contrast used. As measured by digital capnography, there was no sustainable change in pulmonary function in either group during the procedures, except for a slight increase in the 2-3 breaths that followed CO_2 injection, as expected, returning to the initial parameters in all animals.

During the 48-hour observation period, all animals resumed their normal behavior, with regular chow intake and water drinking and no clinical changes as established by the veterinarian. The renal arteries could be correctly identified on all angiographic images. In the subjective analysis of the quality of angiographic images, CO_2 was considered to provide inferior image quality compared to iodinated contrast, without, however, preventing the procedure from being performed. No quantitative method was used for image comparison.

Discussion

The prototype intravascular CO_2 injector tested in the present study was effective in obtaining angiographic vascular images and proved to be suitable for the proposed angiographic procedures, with no need for additional imaging with iodinated contrast to successfully perform the procedures.

The *in vivo* evaluation of a prototype device, even in a small number of animals, allows the detection of possible design errors and issues related to the practical applicability of the prototype that have not been identified *in vitro*. As previously demonstrated *in vitro*, the intravascular CO_2 injector proved to be consistent in delivering scheduled CO_2 injections, with no need to interrupt the procedure due to equipment failure. *In vitro* tests have also shown that the device has achieved adequate accuracy for use in arteriographic studies, since there is a wide margin of safety regarding the volume of CO_2 injected. In the *in vitro* assay, with the gas flow adjusted to 20 mL/s, the difference between the total volume adjusted and total volume injected was less than 10%. This margin of error increased proportionally to higher adjusted volumes, without, however, affecting the intended use of the device, since it is recommended that the device be adjusted at a flow rate of 10 mL/s or 20 mL/s for angiography.

The design of the intravascular CO_2 injector considered the difficulties and risks associated with the use of CO_2. The benefits of electronic microprocessor technology, with highly reliable and portable devices, were employed to overcome the risk of contamination of the delivery system with room air, which was demonstrated by the positive CO_2 flow while the device was powered on. This constant, very small flow of CO_2 assures the device hose is not filled with room air. The apparatus also consists of a one-way valve assembly that allows us to connect the injection hose nozzle to the catheter or introducer, in the same way that has already been used for iodinated contrast injection, without the need for water seal. The sterile extension hose remains connected to the unit throughout the procedure in the sterile surgical field, ready to be used at any time. The microprocessor and valve system ensure the reproducibility of the gas volume at the predetermined flow rate, facilitating image comparison, a goal that cannot be achieved with the same degree of reproducibility by hand injection using a syringe with gas or a plastic reservoir bag.

Medical-grade CO_2 costs about USD 50 for a 5-liter gas cylinder refill, which can be used for many procedures, a reduced cost compared to the USD 60 spent on a 50 mL single-use vial of iodinated contrast (Iodixanol, Visipaque 320 mg/mL, 50 mL vial - GE Healthcare). This difference in the price of the primary product allows the device tested here, with initial fixed cost estimated at USD 5,000, to become economically feasible, with recovery of the initial investment in about 3-5 months, considering a conservative scenario of use of three examinations per day. This information is particularly important when considering the potential use of this device in the public health system, which is the case in Brazil.

The easy handling of the intravascular CO_2 injector allows the procedure to be performed at the same speed as the procedures commonly performed with iodinated contrast injectors, since the delivery system is ready for use throughout the procedure, with continuous supply of CO_2 in the apparatus. The necessary adjustments to the CO_2 injector are similar to those required in the iodinated contrast injectors, that is, total volume of CO_2 (in mL) and injection flow rate (in mL/s). The device also has pre-stored parameters from the most relevant studies, facilitating change of volume flow configuration for angiography. In the currently available hand-delivering methods for CO_2 injection, when using syringes manually loaded under water seal or devices with a plastic reservoir bag, handling is more time consuming, and it is necessary to purge the system when loaded to reduce the risk of room air contamination, compromising agility.

This study has some limitations. The quality of the angiographic images obtained with CO_2 was considered, although subjectively analyzed by investigators involved in the procedures, of inferior quality compared to those obtained with iodinated contrast. However, all the proposed procedures with CO_2 were successfully performed and no technical difficulties were reported in the identification of the renal arteries when the CO_2 injector was used, with a similar number of successfully completed angiographic procedures in both groups (Figures 6,7). The fluoroscopy unit used in the present study dates from the early 1990s and can be considered obsolete, in light of equipment produced nowadays, and this may have been the main cause of inferior image quality reported in the cases using CO_2. Hemodynamic and C-arm systems with updated software already have specific programs for image acquisition using CO_2, optimizing and providing similar image quality with both contrast agents. Even with the drawbacks of a technologically outdated fluoroscopy unit, the resolution of the images produced in this study was satisfactory in all cases, allowing clear identification of the primary and secondary branches of the renal artery.

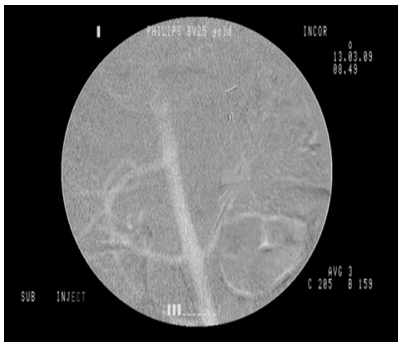

Figure 6: CO_2 angiography, animal 4.

There are currently a few different ways to deliver CO_2 during angiographic procedures, either by hand or by an automated system: hand injection with direct filling of a syringe from an external CO_2 reservoir or hand injection after filling the syringe through one of the 2 devices designed to this purpose: the Angioset device (OptiMed, Ettlingen, Germany) and the Co2mmander (AngioAdvancements, Ft. Meyers, Fla - USA). There are as well automated CO_2 injectors such as the Angiodroid (Angiodroid SRL, San Lazzaro di Savena, Italy) and the Inspect 3005R and 2005R (Malek Medical GmbH, Wismar, Germany).

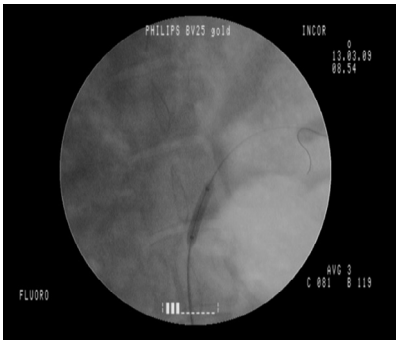

Figure 7: Renal angioplasty, animal 4.

The prototype evaluated here should join the automated class of CO_2 injectors, once it makes its way into the market.

The absence of laboratory abnormalities in the intraoperative tests (serial blood gas analysis) and postoperative tests (serum creatinine) is consistent with published data that demonstrate the safety of CO_2 injection as an angiographic contrast medium. The animals remained clinically and hemodynamically stable both during surgery and in the first 48 hours postoperatively. Within the scope of this initial technical feasibility study, the results suggest proper functioning of the prototype intravascular CO_2 injector.

Conclusion

The prototype intravascular CO_2 injector proved to be feasible for use *in vivo* was effective as the sole source of contrast to perform the proposed arteriographic procedures, allowing successful procedures without unexpected complications, in agreement with the previous positive prototype *in vitro* results. There were no technical failures associated with the use of the prototype system for delivery of CO_2 *in vivo*, and no clinical or arteriographic evidence of contamination of the injected CO_2 with room air. Based on clinical follow-up and laboratory data from serial intraoperative blood gas analyses and pre and postoperative renal function monitoring, the present results demonstrate the initial technical feasibility of the device.

Financial Disclosure

Lanziotti contributed intellectually to the prototype's research and development, but received no royalties or other financial advantages related to it.

Belczak and Silva have no financial relationships relevant to this article to disclose.

Conflict of Interest

Lanziotti contributed intellectually to the prototype's research and development. Belczak and Silva have no conflicts of interest to disclose.

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