The Cerebral Effects of Carbon Dioxide during Digital Subtraction Angiography in the Aortic Arch and Its Branches in Rabbits

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PURPOSE: We studied the neurotoxicity of carbon dioxide as a contrast agent in the central nervous system by performing CO₂ digital subtraction angiography (DSA) in the aortic arch and its branches in experimental animals.

METHODS: Twenty-five rabbits underwent intraarterial CO₂ DSA while under general anesthesia, during which 50 angiograms were obtained after administration of 3 mL/kg CO₂. MR imaging was performed before and after the angiographic procedure. The animals were killed 12 hours later and their brains examined macroscopically and microscopically.

RESULTS: Three animals died of a cause irrelevant to CO₂. No animal had clinical symptoms of hemiplegia or stroke. Neither MR imaging nor macroscopic and microscopic examination of the brain revealed any ischemic infarct hemorrhage, thrombosis, or foci of necrosis.

CONCLUSION: The absence of neurologic symptoms, the lack of pathologic findings at MR imaging, and the negative pathologic findings in the brain encourage further research on CO₂ neurotoxicity of the central nervous system and support its application in the imaging of intracranial vessels.

The use of carbon dioxide as a contrast medium allows the same characterization of radiologic images as do iodinated contrast agents but avoid the possibility of an allergic reaction or renal failure, the frequency of which reaches as high as 20% (1–4). Most experimental and clinical studies have shown that intravenous and intraarterial administration of CO₂ is safe (3–12), except for neurotoxic reactions in the central nervous system (CNS) of mice, caused by damage to the endothelial cell membrane resulting in multifocal ischemic infarcts and neurologic deficits (13). On the other hand, another study with CO₂ administration in the thoracic aorta and the carotid arteries in dogs did not produce any neurologic deficits or electroencephalographic or gross pathologic changes (14).

The present study investigated toxicity in the CNS in rabbits after administration of CO₂ in conjunction with digital subtraction angiography (DSA) in the aortic arch or, selectively, in the carotid and vertebral arteries.

Methods

Twenty rabbits with a mean weight of 2.7 kg were used for this experimental study. An additional five rabbits were used as comparative controls.

The animals were premedicated with 0.5 mg/kg midazolam (Dormicum, Roche, Switzerland) and 0.04 mg/kg fentanyl (Janssen Pharmaceutica, Belgium) intramuscularly. After a marginal ear vein was cannulated, they were anesthetized by intravenous administration of 30 to 40 mg/kg thiopental sodium (Pentothal, Abbott, Italy) as an initial dose, and anesthesia was maintained with supplemental administration of 10% of the above thiopental dose every hour. Cricothyroidotomy was performed, through which an endotracheal uncuffed tube (3-mm internal diameter) was placed into the trachea. Ventilation was controlled manually after the animals were paralyzed with 0.2 mg/kg pancuronium bromide (Pavulon, Organon Teknika, Belgium), followed by additional doses as required. We used a Mapleson C (Mia United Kingdom) rebreathing system in which both fresh gas flow (oxygen in nitrogen; fractional inspired O₂, 0.4 and ventilation 2L/min) were 50% greater than resting minute volume in order to achieve normocapnic conditions (Paco₂ 35 to 45 mm Hg; pH 7.35 to 7.45) (15). A polyethylene catheter (20-gauge) was inserted into an artery for arterial blood gas sampling and hemodynamic measurements. A pulse oximeter with an ear probe was used for arterial oxygen saturation (SaO₂) and pulse rate monitoring. Ringer’s lactate solution was infused at a rate of 4 to 10 mL/kg per hour throughout the experiment.

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We applied near-infrared spectroscopy (Criticon Cerebral RedOx Monitor Model 2020) to determine cerebral oxygenation and hemodynamics in the animals throughout the experiment. Near-infrared spectroscopy provides continuous real-time quantified values for the concentration of oxyhemoglobin (HbO2), deoxyhemoglobin (HHb), and total hemoglobin (tHb) in the tissue. The signal for cytochrome oxidase is not fully quantified, but shows quantified changes in the concentration of the above chromophore (16, 17). Regional oxygen saturation (rSO2) is the percentage of HbO2 in relation to the total hemoglobin (rSO2 = HbO2 / (HbO2 + HHb)) and is automatically calculated by the above monitor. It should be noted that the cerebral tissue under spectroscopy contains not only arterial, but also venous blood, thus rSO2 is a mixed arteriovenous measurement and the value will be lower than that obtained by using a pulse oximeter (SaO2), which estimates only the saturation of the pulsatile (arterial) component of blood flow. Changes in the cerebral blood volume (CBV), which reflect changes in the perfusion state of the brain (18–21), were easily inferred from changes in the tHb concentration.

Before and immediately after each CO2 injection into the aorta, or selectively into the internal carotid artery, blood samples were drawn from the catheterized artery to check pH, PaO2, and PaCO2, and the animal’s blood pressure was monitored invasively.

Following completion of the above procedure, the femoral artery was sectioned and paracentesis of the radial artery was performed with an arrow-type 22-gauge catheter. A hydrophilic 25-inch, 64-mm guidewire (Meadow) was then propelled as far as the aortic arch under fluoroscopic guidance followed by introduction of a pediatric 3F head catheter into the aortic arch or, selectively, into the common carotid or vertebral artery. Periodic administration of 1 to 2 mL of heparinized serum was used to avoid clotting the catheter; also 1 mL of blood was taken to check blood gases. The catheter was subsequently filled with heparinized serum and connected to the system delivering the CO2.

Five control animals were initially used to establish and compare normal parameters of magnetic resonance (MR) imaging and angiography of the aortic arch and its branches performed with typical, iodinated contrast material.

Before infusion, the syringe was coupled to a continuous CO2 flow and the plunger was placed beneath the CO2 flow. The plunger was then joined to a catheter system, which, after each connection with the continuous CO2 flow, was immersed into heparinized serum and drawn as far as the syringe. The continuous flow of CO2 and the closed circuit of catheter-syringe full of serum prevented the introduction of air bubbles. Each injection included 3 mL/kg of pure CO2 injected via a disposable inflation device (Wizard II, USCI), which guaranteed exact control of quantity and approximate control of pressure applied. With the animal in an anepine state, angiograms were obtained at a rate of three per second during a period of 4 seconds. The CO2 was administered with intervals of at least 3 minutes between each dose, while the animal’s head was slightly elevated to 45° to avoid the pooling of bubbles into the arterial system. Angiograms were reviewed by two radiologists, who rated diagnostic accuracy and image quality subjectively on a scale of 1 to 4 as compared with the control animals (1 = excellent, 2 = very good, 3 = good, 4 = bad). MR imaging before and 8 hours after the experiment included axial T1-weighted spin-echo images (450/25/4 [repetition time/echo time/excitations]), coronal T2-weighted images (2000/100/2), and proton density–weighted images (200/25/2) with 5-mm-thick sections, a 25-cm field of view, and a 192 × 192 matrix.

The animals were killed 12 hours later, and the brains of all 25 rabbits were removed, fixed in 10% buffered formalin for 48 hours, and serially cut into 0.5-mm-thick coronal sections. Twenty-four tissue blocks were sampled from each brain, 20 from the cerebrum, three from the cerebellum, and one from the medulla. The 1 × 1 × 0.5-mm tissue blocks were embedded in paraffin, cut at 4 μm, and stained with hematoxylin-eosin.

**Results**

A total of 50 angiographic procedures were carried out, and an average of 29.15 mL of CO2 was given to each experimental animal (Table 1). Thirty-two images of the aortic arch, 14 of the carotid arteries, and four of the vertebral arteries were taken selectively.

During the injection of CO2, no significant changes in arterial blood gases or SaO2 were observed under any volume and pressure conditions we used, whereas the mean arterial pressure increased about 15 to 25 mm Hg soon after the injection and decreased to the preinjection level 1 to 2 minutes later. The pulse rate, indirectly detected from the pulse oximeter, showed a remarkable decrease during and immediately after the CO2 injection. Soon after that, a rise in pulse rate to a higher than preinjection level was observed. Paralysis of the animals prevented us from observing any change in respiratory rate that might have been caused by CO2 injection. However, in a few animals, which during the initial setup of the experiment had not been paralyzed, the intracarotid CO2 injection led at first to slow and deep breaths and later to irregular respiration with random deep and shallow breaths. The near-infrared spectroscopy monitor showed a reduction in tHb at the time of injection, and a simultaneous reduction in rSO2. On the other hand, the immediate postinjection phase was characterized by a marked increase in tHb even beyond the preinjection levels, while rSO2 remained low. Unfortunately, our cytochrome oxidase measurements were insufficient and not of good quality because of a high interference index in the cytochrome oxidase trace. During the period following anesthesia, no signs of hemiplegia or stroke were observed.

DSA afforded good visibility of the aortic arch and its tributaries (Fig 1). Both large and medium-sized arteries were also judged satisfactorily as compared with conventional angiography (Fig 2). At an atmospheric pressure of 1 to 1.5 atm, at which CO2 was administered, imaging of the arterial system enabled concomitant visualization of the aortic arch and its branches as well as the inferior aorta (Fig 3). Imaging of the carotid and vertebral arteries was selectively determined (Fig 4). By consensus, the two interpreters rated all images as either grade 2 (very good) or grade 3 (good).

Three of the experimental animals died (numbers 2, 4, and 15), but these deaths seem to be unrelated to CO2 administration.

Comparative preoperative and postoperative control MR imaging showed an absence of ischemic infarct or hemorrhage during a mean period of 10 hours after CO2 DSA; brain edema only in animals 2 and 4; and, in two other animals (numbers 9 and 10), three to five round black spots approximately 2 mm in diameter, in symmetric sites of both hemispheres. These spots were present with homogeneous imaging of the brain matter, which we consider typical for imaging vessels or cerebrospinal fluid in the subarachnoid space.
<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight, kg</th>
<th>Angiographic Vessel</th>
<th>Quantity of Carbon Dioxide, mL</th>
<th>Pressure, atm</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.2</td>
<td>Aortic arch</td>
<td>15</td>
<td>1</td>
<td>Aortic arch and its branches</td>
</tr>
<tr>
<td>2*</td>
<td>4.3</td>
<td>Aortic arch</td>
<td>15</td>
<td>1</td>
<td>Continuous administration without free interval; somnolence, spasm, death.</td>
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<tr>
<td></td>
<td></td>
<td>Aortic arch and intracranial vessels</td>
<td>12</td>
<td>12</td>
<td></td>
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<tr>
<td>3</td>
<td>3.1</td>
<td>Aortic arch</td>
<td>20</td>
<td>1.5</td>
<td>Aortic arch, L carotid artery, and intracranial vessels</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L carotid artery</td>
<td>5</td>
<td></td>
<td>MR: cerebral edema</td>
</tr>
<tr>
<td>4*</td>
<td>2.8</td>
<td>Aortic arch</td>
<td>15</td>
<td>1.5</td>
<td>Loss of blood, administration of fluids, cerebral edema (established through MR and histology)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L vertebral artery</td>
<td>5</td>
<td></td>
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<tr>
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<td>2.7</td>
<td>Aortic arch</td>
<td>16</td>
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<td>Aortic arch, R carotid artery, and intracranial vessels</td>
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<td></td>
<td>R carotid artery</td>
<td>10</td>
<td></td>
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<tr>
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<td>3.0</td>
<td>L carotid artery</td>
<td>9</td>
<td>1</td>
<td>Selective imaging of L carotid artery and branches of aortic arch</td>
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<td></td>
<td></td>
<td>Aortic arch</td>
<td>9</td>
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<tr>
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<td>3.5</td>
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<td>1</td>
<td>L vertebral artery and L carotid artery. MR: black spots</td>
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<td>L carotid artery</td>
<td>10</td>
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<tr>
<td>10</td>
<td>3.0</td>
<td>L carotid artery</td>
<td>8</td>
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<td>Angiography: L carotid artery and aortic arch with its branches. MR: black spots</td>
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<tr>
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<td>Aortic arch, R carotid artery, L vertebral artery</td>
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<td>Aortic arch and its branches, selectively R carotid and L vertebral artery</td>
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<td>14</td>
<td>2.5</td>
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<td>0.5</td>
<td>Selective imaging of R carotid artery</td>
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<tr>
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<td>1</td>
<td>Respiratory disturbances</td>
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<td>8</td>
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<tr>
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<td>8</td>
<td>1</td>
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<tr>
<td>19</td>
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<td>Aortic arch and selective L carotid artery</td>
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<td>20</td>
<td>3.0</td>
<td>Aortic arch</td>
<td>9</td>
<td>1</td>
<td>Aortic arch with its branches and selective R carotid artery</td>
</tr>
</tbody>
</table>

* These animals died, but the deaths seemed unrelated to CO₂ administration.
Gross examination of the 25 brains showed only slight congestion of the meninges. There was no evidence of any other changes. The vessels were unremarkable, the ventricles contained clear fluid, and there were no foci of necrosis in the brain substance. Microscopic examination showed moderate extracellular edema in the two rabbits (numbers 2 and 4) that died. There was no evidence of thrombosis, hemorrhage, or ischemic necrosis in any of the other rabbits. The brains of the control animals were unremarkable.

**Discussion**

Carbon dioxide was used as a radiologic contrast medium in humans 40 years ago, but it is difficult to administer and the subtle difference in density between the contrast medium in the blood vessels and the surrounding soft tissues requires the use of DSA (22, 23).

In 1982, Hawkins (24) used CO₂ DSA to visualize the splanchnic arteries and arteries in the lower extremities in 20 patients. One year later, Miller et
so that CO₂ might not be tolerated by the CNS of infarctions caused by the hypoxic effect of gas emboli, dothelial cell membranes, and multifocal ischemic produced immediate dramatic neurologic deficits, certain undesirable characteristics of CO₂ (invisibili- ty, susceptibility to air contamination, pooling of gas bubbles) and the possibility of neurotoxicity. The administration of CO₂ in the carotid arteries of mice has produced immediate dramatic neurologic deficits, disruption of the blood-brain barrier and of the endothelial cell membranes, and multifocal ischemic infarctions caused by the hypoxic effect of gas emboli, so that CO₂ might not be tolerated by the CNS of every mammal (13). Other investigators, on the basis of indirect measurements of the cerebral flow and brain electrical activity as end-points, have suggested that ischemia caused by small gas emboli is reversible (22, 23).

These ambiguous findings are probably due to technical weaknesses and to inconsistencies in the experimental models, including the mode of CO₂ administration (pressure, quantity, infusion time); the anatomic significance of the vessels in which the gas bubbles were trapped; and the kind and hemodynamic condition of the experimental animals used, which influence the time of lodging of the gas bubbles within the vessel (23). These data could explain the contradictory results obtained among different experi- mental specimens.

The dramatic and spiked effect of intracarotid CO₂ injection on pulse rate might well be related to a sharp increase in intracranial pressure and is a common clinical sign (similar to the respiratory disturbances in the nonparalyzed animals) in acute intracranial hemorrhage. The near-infrared spectroscopy monitor showed a reduction in rSO₂ at the time of injection, which, in combination with a negative change in CBV, indicated an acute diminution in cerebral blood flow (CBF) caused by the sudden increase in intracranial pressure (25), and resulted in extended HbO₂ desaturation (18).

On the other hand, the immediate postinjection phase was characterized by a marked increase in CBV, even beyond the preinjection levels, while rSO₂ remained low, a situation similar to the postischemic hyperemia observed even after an experimentally induced 30-second cessation of CBF (26, 27). This postocclusive overabundant CBF relative to metabolic needs is caused by a number of vasoactive metabolites generated during ischemia and by the early reperfu- sion period (28, 29). The direct local vasodilatation effect of the injected CO₂ and the slowing of the pulse rate caused by high intracranial pressure may also play a role in reversing the expected hemodynamic consequences described above (30, 31). In other ex- periments, in which systemic hypercapnia was induced either by breathing CO₂ (29) or by hypoventi-
and simultaneously cytotoxic edema. The passage of the CO₂ bubble itself seems to be responsible for the endothelial membrane disruption; however, the precise mechanisms of membrane injury due to CO₂ embolization are not yet clearly understood (13).

Physical deprivation of the liquid-phase contact or the shearing stress on the membrane as the gas-liquid meniscus passes, have also been implicated (13, 45).

Our experimental findings in rabbits, which included an absence of pathologic changes in anatomy or abnormal findings on MR images, as well as the good-quality images we obtained of the aortic arch and its branches, encourage further research on CO₂ neurotoxicity in the CNS and support its application in the imaging of intracranial vessels.

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