Safety of Carbon Dioxide as a Contrast Medium in Cerebral Angiography[1]

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RATIONALE AND OBJECTIVES

An increasing number of studies report the safety and effectiveness of carbon dioxide (CO_2) as a digital subtraction angiographic contrast agent for intraarterial use below the diaphragm. Concerns about the possible neurotoxic effects of CO_2 within the cerebral circulation may be hindering its more widespread use below the diaphragm, because of the potential for reflux into the brain due to its buoyancy. These same concerns have also suppressed any consideration of CO_2 as a potential contrast agent for cerebral angiography.

These concerns have been supported by a study (1) in which rats received single intracarotid injections of CO_2 in a range of doses, causing irreversible blood-brain barrier (BBB) damage and severe neurological deficits, with death occurring within 24 hours at the higher doses. Multifocal ischemic infarction was observed histologically as early as 6 hours after injection (1). Conversely, in a more recent study, dogs received multiple aortic arch and intracarotid injections of CO_2 but showed no EEG changes, no neurological deficits up to 6 months later, and no macroscopic pathological brain changes (2).

There is clearly conflicting evidence about the safety of CO_2 within the cerebral circulation, and this investigation was undertaken with the intention of resolving the issue. Short-term studies were undertaken to determine the effects of intracarotid CO_2 on the BBB, and medium-term studies were undertaken to determine whether any BBB damage was irreversible and whether there was evidence of cerebral infarction.

MATERIALS AND METHODS

New Zealand white rabbits were anesthetized with fentanyl fluanisone (Hypnorm; Janssen, High Wycombe, Buckinghamshire, UK) at 0.4 mL/kg subcutaneously and midazolam (Hypnovel; Roche, Dee Why, N.S.W., Australia), at 2 mg/kg intravenously. All animals were intubated, their left external carotid artery was catheterized retrogradely, and they received an intravenous injection of Evans blue (2%, 3 mL/kg).

In the first (short-term) series of experiments, animals received an intravenous injection of Tc-99m pertechnetate (4–5 MBq in 0.2 mL saline). Experimental animals ($n = 7$) then received five intracarotid injections of medical-grade CO_2 at 2-minute intervals; each injection was for 3 seconds at a rate of 20 mL/min. (This injection rate was determined from preliminary experiments to be the lowest that yielded clinically useful images by digital subtraction angiography.) The CO_2 injections were made with an infusion pump with a controlled, nonexplosive delivery. Control animals ($n = 5$) received five injections of iopromide (Ultravist 300; Schering, Berlin, Germany) at 2-minute intervals at a rate of 5 mL/min for 3 seconds.

Twenty-eight minutes later, horseradish peroxidase (HRP, 50 mg in 1 mL saline) was injected via the carotid catheter. Two minutes after this, the thorax was opened, 1 mL of cardiac blood was removed, and the animal was exsanguinated by transcardiac perfusion with 0.1 M phosphate-buffered 4% formaldehyde and 1% glutaraldehyde. The brain was removed, and the degree of Evans blue staining was scored visually on a scale from 0 to 3+. The Tc-99m activity was determined in both hemispheres and

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in the blood sample. The brain/blood activity ratio was calculated for each hemisphere, and the right (noninjected) hemisphere ratio was subtracted from the left (injected) ratio to give a figure, described as pertechnetate uptake, which estimates the degree of BBB breakdown. Differences in pertechnetate uptake between control and experimental groups were tested by the Student *t* test.

Brains were cut into 5-mm-thick coronal slices. From the most anterior three slices, 40-µm-thick coronal Vibratome sections were cut at 200-µm intervals. Extravasated HRP was visualized in these sections histochemically using the diaminobenzidine (DAB) method, and they were mounted for light microscopy.

In the second (medium-term) series of experiments, animals received an intravenous injection of Tc-99m pertechnetate (12 MBq in 0.2 mL saline) after which experimental ($n = 7$) and control ($n = 5$) animals received five intracarotid injections of CO_2 or iopromide, respectively, under the same conditions as in the first series of experiments. Following the injections, the catheter was withdrawn, the neck wound sutured, and the animals allowed to recover. Six hours later, 50 mg HRP was injected intravenously in 1 mL of saline. Two minutes after this, the thorax was opened, 1 mL of cardiac blood was removed, and the animal was exsanguinated by transcardiac perfusion with either saline ($n = 4$ experimental, $n = 2$ control) or 0.1 M phosphate-buffered 4% formaldehyde and 1% glutaraldehyde ($n = 3$ experimental, $n = 3$ control). The brain was removed and scored for the degree of Evans blue staining, and then pertechnetate uptake was calculated as in the first series of experiments. Differences in pertechnetate uptake between control and experimental groups were tested by the Student *t* test.

Saline-perfused brains were cut into 5-mm-thick coronal slices that were then incubated in 1% 2,3,5-triphenyltetrazolium chloride (TTC; Sigma, St. Louis, Mo) for 15 minutes at 37°C. The slices were examined under a dissecting microscope for uniformity of staining.

Aldehyde-perfused brains were processed for the light microscopic evaluation of HRP extravasation as in the first series of experiments.

RESULTS

In the first series of experiments, all experimental animals showed Evans blue staining of the left (injected) hemisphere (Table 1), with occasional staining of a dorsomedial portion of the right hemisphere. There was no Evans blue staining of either hemisphere in the control animals. The pertechnetate-uptake figure for the control group (mean ± SD) was 0.0005 ± 0.0012, while the figure for the experimental group was 0.0161 ± 0.0081, which was significantly greater ($P < .002$).

Table 1
Short-term Studies: Degree of Left Hemisphere Evans Blue Staining

Group	0	+	++	+++
Control ($n = 5$)	5	–	–	–
Experimental ($n = 7$)	–	5	2	–

Table 2
Medium-term Studies: Degree of Left Hemisphere Evans Blue Staining.

Group	0	+	++	+++
Control ($n = 5$)	5	–	–	–
Experimental ($n = 7$)	–	1	6	–

Light microscopic analysis of the coronal brain sections revealed HRP extravasation around many vessels in the left hemispheres of the experimental group. These were predominantly penetrating arterioles but included some large veins. The HRP was visible as a brown reaction product within the walls of the vessels. No vessels in the control group showed any HRP extravasation, in either hemisphere.

In the second series of experiments, all experimental animals showed Evans blue staining of the injected hemisphere (Table 2), again with occasional staining of a dorsomedial portion of the contralateral hemisphere. The level of staining was slightly greater than in the first series of experiments. There was no staining of either hemisphere in the control animals. The pertechnetate-uptake figure for the control group (mean ± SD) was –0.0017 ± 0.0023, while for the experimental group it was 0.0192 ± 0.0076, which was significantly greater ($P < .0005$).

In the three experimental animals treated to reveal extravasated HRP, no stained vessels were found in one left hemisphere, while one or two stained vessels were found in total in all the sections examined from the other two left hemispheres. No HRP extravasation was observed in vessels from either hemisphere of control animals. In the four experimental animals whose brains were incubated with TTC, all brain slices, from both hemispheres, were uniformly stained brick red, with no unstained areas visible under a dissecting microscope. Brain slices from the control animals were likewise uniformly stained red.

CONCLUSION

In the short-term experiments, the presence of extravasated Evans blue in the injected hemispheres of all experimental rabbits indicates that multiple intracarotid injections of CO_2, at a clinically relevant dose rate, caused opening of the BBB that was not seen in control animals. This was confirmed quantitatively by the finding that there was significantly greater extravasation of pertechnetate (ie, pertechnetate uptake) in the experimental animals than in the control animals. Both Evans blue and pertechnetate were injected intravenously immediately before the CO_2 injections, so that they would have begun to be extravasated from the moment that the BBB was opened. HRP, on the other hand, was injected 28 minutes after the last CO_2 injection and was allowed to circulate for only 2 minutes before the animal was perfused. This short circulation time meant that any extravasated HRP was localized within the walls of individual blood vessels with increased permeability before it could diffuse into the cerebral extracellular space. The light microscopic observation that many vessels from the injected hemispheres of experimental animals had extravasated HRP within their walls indicates that the BBB was still open, at least to some extent, 30 minutes after the last CO_2 injection. It is not possible to know from these experiments, however, whether the BBB had begun to close at this time and whether there would have been still more leaky vessels demonstrated had the HRP been injected immediately after the last CO_2 injection, with perfusion 2 minutes later.

In the medium-term experiments, both Evans blue and pertechnetate were injected intravenously before animals received multiple intracarotid injections of either CO_2 or iopromide. Animals were allowed to survive for 6 hours before receiving intravenous HRP 2 minutes before perfusion with either saline or aldehyde fixative. After 6 hours, all left hemispheres of experimental animals were stained by Evans blue, indicating that BBB breakdown had occurred in all animals as a consequence of the multiple intracarotid CO_2 injections. This was again confirmed by the significantly greater pertechnetate uptake in the experimental animals at 6 hours. The level of Evans blue staining in these experiments was slightly greater than in the short-term experiments, suggesting that the BBB remained open for longer than 30 minutes, thus allowing more Evans blue to extravasate in the period between 30 minutes and 6 hours postinjection. By 6 hours, the BBB opening had reversed, since HRP injected at this time was rarely observed to have extravasated into the walls of cerebral vessels. Examination of TTC-stained brain slices taken 6 hours after intracarotid injection of either CO_2 or iopromide revealed no unstained areas indicative of infarction. As a method of demonstrating cerebral infarction in the rabbit, staining with TTC has been shown to correlate highly with routine hematoxylin-eosin histology (3).

In summary, this study has shown that multiple intracarotid injections of CO_2, at clinically relevant doses, cause initial BBB breakdown which is reversible within 6 hours but do not appear to cause infarcts that are detectable within 6 hours of the injections. The results conflict with those of an earlier study in which rats that received single intracarotid injections of CO_2 showed irreversible BBB breakdown, severe neurological deficits, and multifocal ischemic infarction that was demonstrable histologically at 6 hours after injection (1). The adverse effects in rats may have been due to the relatively large volumes of CO_2 injected or to the explosive manner of its delivery (4).

The study suggests that CO_2 can be used safely as a digital subtraction angiographic contrast agent below the diaphragm, with little risk of severe neurotoxic effects should there be reflux of CO_2 into the cerebral circulation. Although intracarotid CO_2 caused reversible BBB breakdown, it retains potential as a contrast agent for cerebral angiography in some circumstances, since patients undergoing repeat hyperosmotic BBB disruption with intraarterial chemotherapy show no deterioration in cognitive or neurological function (5).

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