The Effects of Carbon Dioxide versus Ioxaglate in the Rat Kidney

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PURPOSE: The renal medulla seems to be particularly vulnerable to vascular injection of iodinated contrast media, particularly in patients with preexisting renal dysfunction. The gas carbon dioxide is frequently used as an alternative to iodinated contrast medium in these patients. In this study, the renal effects of CO2 are investigated and compared with those of the iodinated contrast medium ioxaglate.

MATERIALS AND METHODS: Cortical and outer medullary blood flow (measured by laser Doppler flowmetry) and oxygen tension (Po2; measured by oxygen microelectrodes) were recorded in anesthetized Sprague-Dawley rats given an intraarterial injection of ioxaglate (320 mgI/kg body weight), a volume-matched dose of CO2, or Ringer solution.

RESULTS: Injection of CO2 induced a pronounced and transient decrease in cortical blood flow and Po2 (approximately −45%), whereas outer medullary blood flow and Po2 were transiently increased (+21% and +29%, respectively). In contrast, injection of ioxaglate did not influence cortical blood flow and caused outer medullary blood flow to decrease by 17%. Ioxaglate injection also resulted in a decrease in cortical and outer medullary Po2 (−15% and −33%, respectively). Ringer solution affected none of the recorded parameters.

CONCLUSIONS: Although injection of CO2 markedly affected regional renal blood flow and Po2, there were qualitatively different effects in the cortex and outer medulla compared with those seen after injection of ioxaglate. The pronounced decrease in medullary blood flow and Po2 observed after injection of ioxaglate was absent in the animals injected with CO2. This might suggest beneficial effects of the use of CO2 instead of iodinated contrast media in patients with increased risk of developing renal failure.

The gas carbon dioxide was used in subtraction angiography for the first time in the 1950s (1). In recent years, the replacement of iodinated contrast media by CO2 has increased in the performance of angiography in patients with reduced renal function (2). Signs of reduced renal function include reduced glomerular filtration rate (GFR) and subsequent increase in serum creatinine concentration. These patients have an increased risk of developing iodinated contrast media–induced nephropathy (3). In a recent study, Liss and coworkers (4) found that the risk for impaired renal function in patients after replacement of iodinated contrast medium by CO2 is lower than after injection of iodinated contrast medium alone. The exact mechanism for the increased risk of contrast media–induced nephropathy in patients with reduced renal function is not fully understood. Renal medullary hypoxia (5) and increased renal proximal tubular oxygen consumption (6) have been proposed as possible mechanisms, among numerous other hypotheses. In previous studies in the rat (7,8), we have shown marked effects on local renal hemodynamics and oxygen tension (Po2) after administration of iodinated contrast medium. The renal medulla is especially likely to be exposed to decreased Po2 after contrast medium administration (8).

In this study, we investigated the effects of an injection of CO2 into the renal artery on local renal blood flow and Po2 in rats. The renal effects of CO2 were compared with those recorded after a similar injection of the low-osmolar ionic contrast medium ioxaglate. The aim was to elucidate possible differences in renal effector mechanisms between CO2 and ioxaglate to explain previous findings in patients (4).
MATERIALS AND METHODS

Animals

The experiments were performed on 22 adult male Sprague-Dawley rats, which were purchased from M&B Research and Breeding Center (Ry, Denmark). The animals weighed 260–320 g and had free access to water and standard rat chow (R3; Ewos, Södertälje, Sweden) throughout the study. The local animal ethics committee approved all experiments.

Experimental Groups

The animals were divided into three groups that received CO₂ (n = 9), ioxaglate (n = 6), or volume-matched Ringer solution (n = 7) intravenously. Inclusion of six to nine animals in each experimental group has previously been shown to be sufficient to detect physiologically relevant differences in similar studies. Each measurement made in the present study was performed once in each animal.

Surgical Procedures

The animals were anesthetized with an intraperitoneal injection of thiobutabarbital (Inactin; Research Biochemicals International, Natick, MA; 120 mg/kg body weight), placed on an operating table, and tracheostomized, and the body temperature was maintained at 37°C. Polyethylene catheters were placed in the left femoral artery, left femoral vein, and aorta. The catheter in the femoral artery was used for monitoring blood pressure (P23db; Statham Laboratories, Irvine, CA) and for blood sampling. The femoral vein catheter was used for the injection of the contrast medium. After each experiment, a blood sample was collected for analysis of hematocrit. Animals with mean arterial blood pressures less than 85 mm Hg or hemocrit levels 40% were excluded. All parameters were continuously recorded with a MacLab Instrument (AD Instruments, Hastings, UK) connected to a Macintosh PowerPC 6100 (Apple Computer, Cupertino, CA).

Experimental Protocol

After the surgical procedures, the animals were allowed a 60-minute recovery period. After an additional 20 minutes of control measurements, 0.40 mL contrast medium (ioxaglate; Hexabrix, 320 mgI/mL; Schering, Berlin, Germany), an equivalent volume of CO₂ (at atmospheric pressure), or Ringer solution was injected over a period of 7–10 seconds. The kidney was visually inspected during the injection of the substance.

Statistical Analysis

All values are given as means ± SEM. When two sets of data within the same experimental group were compared, a Student paired t test was applied. Comparisons with baseline were performed at the time when the greatest change was recorded. Between groups with different treatments, analysis of variance followed by Fisher protected least significant differences test were used. For comparison of repeated measurements within the same group, analysis of variance followed by a post-hoc test

Po₂ in the renal cortex and renal medulla was measured with a polarographic technique using modified Clark-type microelectrodes (Unise, Aarhus, Denmark) (9) applied to the kidney as previously described (7). A linear correlation was obtained between Po₂ and electric current. The latter was measured by picocompmeters (University of Aarhus, Denmark). The electrodes were calibrated in water saturated with Na₂S₂O₃ or water bubbled with air at 37°C. They were inserted into the renal cortex and outer medulla by the use of a micromanipulator under a stereomicroscope. The renal cortical and outer medullary blood flows were measured with laser Doppler flowmetry (PF 4001–2; Perimed, Stockholm, Sweden). One laser Doppler probe was placed on the renal surface to record cortical blood flow. A second needle probe (tip 0.45 mm outer diameter; 411; Perimed) was inserted into the outer renal medulla with use of a micromanipulator for simultaneous recording of the outer medullary blood flow. Simultaneous measurement of Po₂ and blood perfusion in the renal cortex and renal medulla has previously been shown to be a useful method for detection of alterations in oxygen metabolism and microcirculation (10). After the experiment, the kidney was dissected to verify the site of Po₂ and blood flow measurements. If any site of measurement was found not to be correctly located, the recording was excluded. All parameters were continuously recorded with a MacLab Instrument (AD Instruments, Hastings, UK) connected to a Macintosh Power-PC 6100 (Apple Computer, Cupertino, CA).
for repeated measurements (Statview; Abacus Concepts, Berkeley, CA) was applied. For all comparisons, absolute values and not percentage values were used. A P value less than .05 was considered statistically significant.

RESULTS

Body Weight, Mean Arterial Blood Pressure, GFR, and Urinary Parameters

The rats (n = 22) weighed 285 g ± 4 and there was no difference among the experimental groups (Table). The mean arterial blood pressure was unaffected by injection of ioxaglate or Ringer solution, whereas an injection of CO₂ resulted in transient blood pressure increase. The baseline mean arterial pressure was slightly lower in the CO₂-injected animals compared with the other two groups. GFR was unchanged by injection of Ringer solution and increased after injection of ioxaglate, whereas CO₂ injection transiently reduced the GFR. In all groups, the urinary flow rate and excretion of osmotically active particles increased after injection. However, this was accompanied by increased sodium excretion in only the ioxaglate-treated group.

Cortical Blood Flow and Po₂

During the control period, absolute cortical renal blood flow (Fig 1) was similar in all investigated groups (330 laser units ± 2, 295 laser units ± 14, and 301 laser units ± 33 for the animals later injected with CO₂, ioxaglate, and Ringer solution, respectively). Likewise, renal cortical Po₂ (Fig 2) did not differ among animals in the different experimental groups before injection (27.5 mm Hg ± 0.8, 28.7 mm Hg ± 3.2, and 26.6 ± 0.8 in the CO₂, ioxaglate, and Ringer solution groups, respectively). Renal cortical blood flow was not affected by injection of ioxaglate, whereas injection of CO₂ resulted in transiently reduced cortical perfusion. Injection of ioxaglate as well as CO₂ decreased the cortical Po₂. The injection of volume-matched Ringer solution did not affect blood flow or Po₂ in the renal cortex.
Before the respective injections, outer medullary blood flow (Fig 3) differed among the three experimental groups. The animals that later received an injection of CO₂ had higher blood perfusion (131 laser units \pm 8) than the animals later injected with ioxaglate (100 laser units \pm 4) or Ringer solution (110 laser units \pm 3; \( P < .05 \) for both comparisons). However, there were no differences in outer medullary PO₂ (Fig 4) among the experimental groups before the respective injections (17.6 mm Hg \pm 0.9, 20.6 mm Hg \pm 2.5, and 18.7 mm Hg \pm 1.0 for the animals receiving CO₂, ioxaglate, and Ringer solution, respectively). Ioxaglate injection transiently decreased the renal outer medullary blood flow, whereas CO₂ injection instead resulted in a transient increase. Injection of volume-matched Ringer solution had no effect on outer medullary blood perfusion. Renal medullary PO₂ was unaffected by Ringer solution and decreased after ioxaglate injection, whereas CO₂ injection resulted in a transient hyperoxic response.

A marked and transient whitening of the kidneys injected with CO₂ was observed. No visual effects could be seen during injections of either of the other substances.

Blood Gas Parameters in the CO₂-injected Animals

Before and after injection of CO₂, the arterial blood pH measurements were 7.38 \pm 0.04 and 7.35 \pm 0.01, respectively; arterial PO₂ measurements were 11.2 kPa \pm 0.5 and 10.8 kPa \pm 0.4, respectively; and arterial CO₂ tension measurements were 6.8 kPa \pm 0.4 and 6.7 kPa \pm 0.2, respectively (\( n = 9 \)). None of these measurements before
and after CO₂ injection were statistically different. No measurements of blood gas parameters were performed in the other groups.

DISCUSSION

The use of contrast medium in diagnostic and interventional procedures has increased during recent decades. The frequent use of contrast medium also results in increasing numbers of contrast media–induced nephropathy (11). The two major factors contributing to a higher risk of developing contrast media–induced nephropathy are diabetes and preexisting renal impairment (12). Patients with increased risk of developing contrast media–induced nephropathy also belong to a large group of patients in whom radiographic procedures are needed, and the prevalence for contrast media–induced nephropathy varies from less than 5% to as high as 50% depending on the risk profile (12,13).

The exact mechanism that accounts for the increased risk of contrast media–induced nephropathy has not been determined, but it has been suggested that medullary hypoxia is a crucial factor for the onset of contrast media–induced nephropathy (5). Consistent with this hypothesis and previous studies (5,10), injection of ioxaglate in the present study decreased the medullary blood flow and Po₂. The reason for the decrease in medullary blood flow and Po₂ after injection of iodinated contrast medium is not fully understood, but several mechanisms have been suggested to be involved. Local changes in adenosine, nitric oxide, or endothelin concentrations are some of these (14–17), but there are conflicting data on the importance of all of these factors (12,18). The increased inulin clearance obtained after injection of ioxaglate has previously been ascribed to a washout effect caused by the momentary increase in urinary flow rate (19). The increased urinary flow rate also contributes in increasing the excretion of sodium and osmotically active particles, as observed in the present study.

In a recent study, Liss and coworkers (4) found that the risk of impaired renal function in patients after replacement of iodinated contrast medium by CO₂ is less than that after injection of iodinated contrast medium alone. There are several advantages of the use of CO₂ instead of conventional iodinated contrast media. Because of the rapid clearance of CO₂ in the lungs, which prevents recirculation, the renal metabolism is likely to be minimally affected (2). Iodinated agents, conversely, depend on renal clearance, which results in an increased load in addition to an often preexisting renal impairment (12). In addition, a main feature of kidney function is concentration of the urine along the tubular system, which results in the medullary cells being exposed to increased concentrations of iodinated contrast medium. Another advantage of the use of CO₂ is that, in contrast to iodinated contrast agents, there is no risk of allergic reactions (2,20). However, a major drawback of the use of CO₂ is the lower contrast properties that the gas provides compared with iodinated contrast agents. Another undesirable effect is that some patients experience nausea after CO₂ injection (21).

A clear visual effect we observed when CO₂ was injected into the renal artery was an immediate whitening of the kidney, which occurred concomitantly with a decrease in cortical blood flow measured by laser Doppler flowmetry on the renal surface. The whitening of the renal surface and concomitant decrease in blood flow were most likely caused by replacement of the blood by the injected gas. This state lasted for approximately 30–40 seconds. Thereafter, the normal renal color returned and the cortical blood flow recovered. The cortical Po₂ also decreased after CO₂ injection and was most probably an effect of decreased blood flow, thereby decreasing oxygen delivery to the tissue. The initial lack of blood in the renal cortex after CO₂ injection can also explain the decrease in GFR. It is highly likely that the filtration of plasma over the glomerular membrane was momentarily stopped when all the blood was substituted by the gas.

Interestingly, intraarterially injected CO₂ caused an increase in renal medullary blood flow and Po₂, which is contrary to the decreased blood perfusion and Po₂ observed after injection of iodinated contrast medium. The blood perfusion of the renal medulla is derived from arterioles supplying juxtamedullary nephrons. These are fewer in number and more deeply situated than the majority of arterioles, which supply the superficial cortical nephrons. Previous investigators have shown that the interlobular arteries act as resistance vessels, resulting in different starting pressures at the beginning of the two types of arterioles supplying cortical or juxtamedullary glomeruli (22,23). The higher resistance and fewer numbers of juxtamedullary arterioles are likely to cause an accumulation of CO₂ preferentially in the renal cortex, leading to a redistribution of blood to the medullary structures, with a concomitant increase in Po₂. The results of the present study add to the body of work available, which localizes changes related to iodinated contrast agents to the renal medulla (5,7,8,24).

Coincidently, there was a higher medullary perfusion in the CO₂ group compared with the other groups during the control period, ie, before CO₂ injection. The reason for this is not known, but it does not influence our conclusions.

In conclusion, injection of CO₂ into the renal artery of normal rats increased the renal medullary blood perfusion and Po₂, which is opposite of the negative effects of the iodinated contrast medium ioxaglate on medullary perfusion and oxygenation. If the same redistribution of intrarenal blood flow occurs in patients with diabetic nephropathy or other preexisting renal impairments, replacement of conventional iodinated contrast medium with CO₂ may decrease the frequency of contrast media–induced nephropathy, provided that the optical contrast properties are sufficient.

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