Peritoneal dialysis impairs nitric oxide homeostasis and may predispose infants with low systolic blood pressure to cerebral ischemia

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ARTICLE INFO
Article history:
Received 6 April 2016
Received in revised form 5 May 2016
Accepted 14 May 2016
Available online 24 May 2016

Keywords:
Blood pressure
Cerebral ischemia
Chronic kidney disease (CKD)
cGMP
Dialysis
Nitrate
Nitrite
Nitric oxide
Nitric oxide synthase (NOS)

ABSTRACT

Background & purpose: Infants on chronic peritoneal dialysis (PD) have an increased risk of developing neurological morbidities; however, the underlying biological mechanisms are poorly understood. In this clinical study, we investigated whether PD-mediated impairment of nitric oxide (NO) bioavailability and signaling, in patients with persistently low systolic blood pressure (SBP), can explain the occurrence of cerebral ischemia.

Methods & results: Repeated blood pressure measurements, serial neuroimaging studies, and investigations of systemic nitrate and nitrite levels, as well as NO signaling, were performed in ten pediatric patients on PD. We consistently observed the loss of both inorganic nitrate (−17 ± 3%, P < 0.05) and nitrite (−34 ± 4%, P < 0.05) during PD, which may result in impairment of the nitrate-nitrite-NO pathway. Indeed, PD was associated with significant reduction of cyclic guanosine monophosphate levels (−59.4 ± 15%, P < 0.05). This reduction in NO signaling was partly prevented by using a commercially available PD solution supplemented with L-arginine. Although PD compromised nitrate-nitrite-NO signaling in all cases, only infants with persistently low SBP developed ischemic cerebral complications.

Conclusions: Our data suggests that PD impairs NO homeostasis and predisposes infants with persistently low SBP to cerebral ischemia. These findings improve current understanding of the pathogenesis of infantile cerebral ischemia induced by PD and may lead to the new treatment strategies to reduce neurological morbidities.

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1. Introduction

The initiation of chronic peritoneal dialysis (PD) during the neonatal period is often associated with an increased risk of developing a variety of major survival limiting morbidities, thus raising the ethical question as to whether chronic PD should be offered to these patients [1]. Neurological complications are a serious concern in this high-risk patient population and account for
considerable mortality [2], however the underlying mechanisms have yet to be elucidated. Efficient autoregulation of blood flow is crucial to maintain adequate tissue perfusion and abnormal regulation has been described in both experimental disease models and in patients with renal and cardiovascular disease [3]. One important modulator of vascular homeostasis and autoregulation is endothelium-derived nitric oxide (NO). Several reports have shown that abnormal NO signaling, particularly within the blood vessel wall, is associated with severe cardiovascular and neurological events (e.g. ischemic stroke) [4–6].

In adult patients on renal replacement treatment, reduced post-hemodialysis plasma nitrate and nitrite levels, which may abrogate the nitrate-nitrite-NO pathway, have been associated with an increased risk for cardiovascular morbidity and mortality [7]. Conversely, there are no pediatric data regarding the effect of chronic renal replacement treatment or PD on nitrate and nitrite anions. We recently inferred that chronic low systolic blood pressure (SBP) coupled with a reduction in nitric oxide (NO) bioavailability, could impair the autoregulation of cerebral blood flow (CBF), thus concomitantly increasing the risk of cerebral ischemia in infants on chronic PD [8]. In the current multicenter study we established a link between persistently low SBP coupled with impaired NO bioavailability and the impairment of cerebral circulation in chronic dialyzed infants.

2. Material and methods

2.1. Study population

All centers in Sweden with expertise and trained personnel in pediatric PD, i.e., the Departments of Pediatric Nephrology at Karolinska University Hospital, at Queen Silvia Children’s Hospital, and at Lund University Hospital participated in this investigation. The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Approval for this multicenter study was obtained from the three local Ethics Committees for human investigations (Protocol number 2014/1631-31). Ten patients (3 males) were included, which are referred in the text in Arabic numerals. At the time of baseline examination, their median age was 0.22 years (range 0.01–13.7) and the median follow-up at the time of writing was 9 months (range 3–22). Informed written consent was obtained from all parents.

Since the main objective of the study was to explore the presumed causal link between persistently low SBP in combination with impaired NO bioavailability and the occurrence of neurological complications in infants on chronic PD the inclusion criteria were chosen to detect neonates on PD that could be followed during the neonatal period throughout infancy. In addition, a secondary objective of the study was to investigate whether chronic PD beyond infancy is also associated with lowering of plasma nitrate and nitrite levels and impairment of the nitrate-nitrite-NO pathway for NO generation. Consequently, inclusion criteria were patients between 0 and 17 years of age on PD. The exclusion criteria were (i) congenital anomalies of the brain and (ii) to be clinically dehydrated or present with an ongoing clinical concern of infection at baseline as well as at repeated examinations.

2.2. Neuroimaging

All neonates and infants underwent a neuroimaging assessment to confirm the presence or absence of ischemic lesions. Decision to repeat neuroimaging was made by the attending physician, and was based on results of previous neuroimaging or on clinical grounds. All neuroimaging was interpreted by an experienced pediatric neuroradiologist (C.C.) who was blinded to the clinical status of the patient. The study protocol did not require any modification of existing PD treatment.

2.3. Peritoneal dialysis

In patients that commenced renal replacement treatment during the neonatal period, i.e., patients 1, 2, 3, 4, 5, and 6, PD was started at the median age of 2 days of life (range 2–23). PD in patients started during the neonatal period was performed using a surgically placed Tenckhoff catheter with partial omentectomy and performed manually with a gravity-based closed exchange system utilizing buretrol to measure fill and drainage volumes. The initial PD prescription consisted of a median fill volume of 10 ml/kg (range 6–14 ml/kg) that was gradually increased to approximately 20–30 ml/kg. The median dwell time was 20 min (range 10–35 min). The fill volume, dwell time, and glucose concentration of dialysate was regularly adjusted according to the individual needs of the patient. Starting PD duration was 24 h and was successively shortened to ~16 h per daily session [9]. Patient 7 was on nocturnal intermittent PD and patient 8, 9, and 10 were on continuous cycling PD, which included a median of 14 cycles (range 10–22 cycles) of 677 ml/m² (range 555–702 ml/m²) of a glucose-based PD solution buffered with bicarbonate/lactate for a median treatment duration time of 12 h (range 10–12 h). In patients 8, 9 and 10 this was followed by a long icodextrin (Extraneal® Baxter Healthcare)-containing daytime dwell.

2.4. Blood pressure measurements

Indirect, non-invasive oscillometric SBP measurements were repeatedly recorded in all participants either during their hospital stay or in the outpatient clinic on the upper right arm in lying position in infants or sitting after a 5–10 min rest period in older children according to recommendations from National High Blood Pressure Education Program (NHBPEP) working group on blood pressure in children and adolescents [10]. In infants that underwent repeated neuroimaging, all SBP recordings that were obtained over four weeks following “baseline examination” were averaged and compared against the age- and sex-related reference values [10].

2.5. Collection and analyses of plasma and PD solution

The total spent dialysate volume, i.e., the total infused dialysate plus ultrafiltrate, was analyzed for nitrate and nitrite, either in a single (patients 5, 6, 7, 8, 9, and 10) or in repeated occasions (patient 1, n = 4; patient 2, n = 6; patient 3, n = 2; patient 4, n = 3). In each patient, the first analysis is regarded as the “baseline examination”. These analyses were performed while the patients were on PD performed as per standard practice using glucose-based PD solution buffered with bicarbonate/lactate. In all occasions, blood samples (2 ml) with EDTA (5 mmol/L) were obtained both at the beginning and at the end of each daily PD session. Blood samples were immediately centrifuged at 1500 g (10 min, 4 °C), and the collected plasma (approx. 1 ml) and dialysate (5 ml) samples were instantly frozen and stored (−20 °C) for later analyses. In addition, patients 1, 2, 4, and 8 underwent a single 4-h dwell that was performed at the end of their standard daily PD session, using a PD solution containing amino-acids including L-Arginine (Nutrineal® Baxter Healthcare). Again, plasma samples were obtained both at the beginning and at the end of each single 4-h dwell. Methods for analyzing nitrate, nitrite and NO signaling, as well as different amino acids, are described below.

Nitrate and nitrite: Similar to that previously described [11,12], a
detailed HPLC system (ENO-20) and auto-sampler (840, EiCom, Kyoto, Japan) was used to measure nitrate and nitrite levels in plasma and dialysate samples. Briefly, the samples were extracted using methanol (1:2) and then centrifuged at 10000 g (10 min, 4 °C). Nitrate and nitrite were separated by reverse phase/ion exchange chromatography followed by nitrate reduction to nitrite by cadmium and reduced copper. The nitrite was then derivatized using Griess reagent to form diazo compounds and analyzed by detection at 540 nm.

**cGMP:** Plasma for cGMP measurements was collected in IBMX-containing tubes (10 μM). Samples were analyzed using cGMP ELISA kit (GE Healthcare, Uppsala, Sweden) according to the manufacturer’s instructions.

**Amino Acids:** Urea cycle amino acids and methyl-arginines were analyzed in both plasma and dialysate, as previously described [13,14]. Briefly, after thawing samples on ice, 25 μl of plasma or dialysate solution were crashed with 225 μl of 0.2% formic acid in isopropanol containing the internal standard (0.73 μmol/L of N4-Arginine). Afterwards samples were vortexed for 30 s and centrifuged at 10000 g (10 min). Finally, 5 μl of the supernatant were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Separation was performed on an ACQUITY UPLC System from Waters Corporation (Milford, MA, USA) using an Atlantis HILIC Silica 3 μm (150 × 2.1 mm) column from Waters. Mobile phases consisted of 0.2% formic acid in ACN:MeOH (75:25 v/v) and 0.2% formic acid in water. Flow rate was set at 400 μl/min. Detection was performed using a Waters Xevo® TQ triple quadrupole equipped with an Electrospray Ion Source working in positive mode. For quantification, the following selected reaction monitoring transitions were used: arginine (175.1 → 701), ornithine (133.1 → 701), citrulline (176.1 → 701), ADMA (203.1 → 460), SDMA (203.1 → 1721), MNMA (189.1 → 701) and N4-arginine (179.1 → 711).

### 2.6. Statistical methods

Comparison of two groups was calculated by Student’s paired t-test. For multiple comparisons, repeated measures ANOVA followed by Holm-Sidak’s test was used. All statistical calculations were made using Graphpad Prism (6.04, La Jolla, CA, USA). Values are presented as mean ± SEM, unless otherwise indicated. Statistical significance was defined as P < 0.05.

### 3. Results

#### 3.1. Study population

The main characteristics of the study population observed at baseline examination as well as the clinical outcome are presented in Table 1. Results on representative single and on serial neuroimaging are shown in Fig. 1 and Fig. 2. In patients 1 and 2, the first neuroimaging was performed 4 days and 74 days before baseline examination whereas in patients 3 and 4, the first neuroimaging was performed 21 days and 1 day after baseline examination, respectively.

In patient 3, progressive ischemic lesions were observed (Fig. 1). Her SBP values were persistently under the 50th percentile for age [10]: median (n = 62) 80 (range 51–101) mmHg. In addition, abnormal neuroimaging was observed in patients 1 and 2. Their SBP values were also under the 50th percentile for age [10]; patient 1: median (n = 91) 86 (range, 47–117) mmHg; patient 2: median (n = 53) 81 (range 69–113) mmHg.

In patient 4, in whom we deliberately avoided overtreatment and consequently excessively low SBP values, her first and second neuroimaging were normal (Fig. 1). Her SBP values were greater than the 95th percentile for age; median (n = 42) 102 (range 83–135) mmHg [10]. Her third neuroimaging, which also was

### Table 1

<table>
<thead>
<tr>
<th>Patient no(M/F)</th>
<th>Age(y)</th>
<th>Underlying diagnosis</th>
<th>Weight(kg)</th>
<th>Height(cm)</th>
<th>BSA(m²)</th>
<th>SBP (mmHg)</th>
<th>P-Na (mmol/L)</th>
<th>P-Alb (g/L)</th>
<th>Comorbidities</th>
<th>Clinical outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(M)</td>
<td>0.18</td>
<td>CAKUT</td>
<td>3.2</td>
<td>52.5</td>
<td>0.21</td>
<td>69</td>
<td>133</td>
<td>23</td>
<td>Anuria, pulmonary hypoplasia, pulmonary hypertension</td>
<td>CCPD commenced at age of 0.32 y</td>
</tr>
<tr>
<td>2(F)</td>
<td>0.27</td>
<td>Renal tubular dysgenesis verified by ACE mutation analysis</td>
<td>4.1</td>
<td>55.5</td>
<td>0.25</td>
<td>67</td>
<td>144</td>
<td>26</td>
<td>Pulmonary hypoplasia, pulmonary hypertension</td>
<td>CCPD commenced at age of 0.36 y</td>
</tr>
<tr>
<td>3(F)</td>
<td>0.01</td>
<td>Autosomal recessive polycystic kidney disease verified by PHKDD mutation analysis</td>
<td>2.2</td>
<td>45.0</td>
<td>0.16</td>
<td>50</td>
<td>132</td>
<td>22</td>
<td>Anuria, pulmonary hypoplasia</td>
<td>Died at age of 0.3 y</td>
</tr>
<tr>
<td>4(F)</td>
<td>0.08</td>
<td>Congenital nephrotic syndrome verified by WT1 mutation analysis</td>
<td>3.3</td>
<td>51.5</td>
<td>0.22</td>
<td>109</td>
<td>140</td>
<td>24</td>
<td>Anuria, pulmonary valve stenosis</td>
<td>On antihypertensive medication. CCPD commenced at age of 0.36 y</td>
</tr>
<tr>
<td>5(M)</td>
<td>0.01</td>
<td>Acute kidney injury</td>
<td>5.0</td>
<td>54.0</td>
<td>0.28</td>
<td>96</td>
<td>127</td>
<td>28</td>
<td>Perinatal asphyxia with multiorgan failure involving mainly the kidney (anuria), lungs &amp; liver</td>
<td>Recovered renal function at age of 0.05 y</td>
</tr>
<tr>
<td>6(M)</td>
<td>0.07</td>
<td>CAKUT</td>
<td>3.0</td>
<td>50.0</td>
<td>0.20</td>
<td>101</td>
<td>133</td>
<td>19</td>
<td>Pulmonary hypoplasia</td>
<td>CCPD commenced at age of 0.24 y</td>
</tr>
<tr>
<td>7(F)</td>
<td>13.7</td>
<td>CAKUT</td>
<td>4.9</td>
<td>156.0</td>
<td>1.39</td>
<td>158</td>
<td>137</td>
<td>25</td>
<td>–</td>
<td>On antihypertensive medication</td>
</tr>
<tr>
<td>8(F)</td>
<td>9.1</td>
<td>Nephronphthisis</td>
<td>23.6</td>
<td>125.7</td>
<td>0.90</td>
<td>114</td>
<td>138</td>
<td>35</td>
<td>–</td>
<td>Renal transplantation at age of 9.2 y</td>
</tr>
<tr>
<td>9(F)</td>
<td>2.2</td>
<td>Congenital nephrotic syndrome verified by NPHS1 mutation analysis</td>
<td>12.7</td>
<td>81.0</td>
<td>0.54</td>
<td>106</td>
<td>146</td>
<td>32</td>
<td>Anuria</td>
<td>On antihypertensive medication, bilateral nephrectomy of the native kidneys at the age of 1.2 y, renal transplantation at age of 2.23 y</td>
</tr>
<tr>
<td>10(F)</td>
<td>1.6</td>
<td>Congenital nephrotic syndrome with negative results of genetic testing</td>
<td>12.0</td>
<td>79.2</td>
<td>0.52</td>
<td>104</td>
<td>140</td>
<td>32</td>
<td>Anuria</td>
<td>Renal transplantation at age of 1.92 y</td>
</tr>
</tbody>
</table>

**Abbreviations:** y, years; M, male; F, female; BSA, body surface area; SBP, average of three systolic blood pressure measurements; P-Na, plasma sodium; P-Alb, plasma albumin; CAKUT, congenital anomalies of the kidney and urinary tract; CCPD, continuous cycling peritoneal dialysis.

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normal, was performed subsequent to a septic event. In patient 5, the neuroimaging results were consistent with hypoxic ischemic encephalopathy preceded by sepsis. Patient 9 developed acute generalized afebrile seizure when admitted at the age of 1.5 years because of severe dehydration due to acute diarrhea while on PD.

Fig. 2 shows imaging sequelae of the brain after her ischemic event. The neuroimaging in patient 8 and 10 was performed as part of the routine workup of kidney recipients. Neuroimaging in patient 10 revealed pathological findings in keeping with cerebral atrophy. In patients 6 and 8, neuroimaging studies were normal. Neuroimaging was not performed in patient 7. In patients with abnormal neuroimaging the neurological outcomes at last follow-up were as follows: patient 1 has microcephaly, but normal gross motor development; patient 2 has microcephaly, with the presence of slightly retarded motor development, and mild hypotonia; patient 3 died at the age of 0.3 years after parents and physicians made the decision to withdraw treatment; patient 9 and 10 have no measurable neurological deficit.

3.2. Peritoneal dialysis with standard glucose-based PD solution

3.2.1. Markers in plasma and dialysate

We investigated whether PD, by limiting the substrates for NO generation, resulted in impaired NO signaling. In agreement with our previous case report, reduced levels of circulating nitrate and nitrite were consistently observed at the end of each PD session (Fig. 3, panel A and B), with plasma nitrite being reduced by $34 \pm 4\%$. Concomitantly there was an increased concentration of these inorganic anions in the dialysate (Fig. 3, panel C and D), demonstrating that nitrate and nitrite are removed during PD (Fig. 3, panel E and F).

The reduction of nitrate and nitrite, which are the substrates for the nitrate-nitrite-NO pathway, may lead to lower NO bioavailability. Indeed a significant reduction in the levels of cyclic guanosine monophosphate (cGMP), a downstream product of NO pathway, was observed ($-59.4 \pm 15\%$, $P = 0.006$) immediately following the PD sessions (Fig. 4, panel A and B), indicating
Fig. 2. Representative neuroimaging performed in patients that initiated peritoneal dialysis after infancy. Pat. 8: FLAIR-MRI sequence showing normal findings. Pat. 9: T2WI-MRI showing extensive gliosis of the periventricular white matter in the frontal lobes and the left parietal lobe as well as small areas of cortical encephalomalacia in the left parietal lobe (arrows). Pat. 10: DWI-MRI showing prominent cerebral sulci and ventriculomegaly (arrows). Pat. indicates patient; Month/year; MRI, magnetic resonance imaging; WI, weighted images; DWI, diffusion weighted images; FLAIR, fluid attenuation inversion recovery.

Fig. 3. Nitrate and nitrite in plasma and dialysate. Analysis of nitrate and nitrite content in plasma, both at the beginning (before) and end (after) of each daily PD session with standard glucose-based PD solution (Glu-based PD), Panel A and B, respectively, and in total spent dialysate volume (dialysate) of each daily PD session (Panel C and D, respectively). Average change of nitrate and nitrite in plasma and dialysate are shown in Panel E and F, respectively. Values are presented as mean ± SEM.
impairment of NO signaling. Of note, dialysis-mediated reduction in the concentration of plasma nitrate, nitrite and cGMP could also be linked to compromised NOS function, due to limitation of the substrate L-arginine. However, plasma levels of arginine, citrulline and ornithine remained unchanged during the PD session (Fig. 5, panel A). Plasma citrulline-to-arginine ratios, a surrogate measure of endothelial NOS activity, were similar at the beginning and at the end of the PD sessions. In addition no changes in plasma ornithine-to-citrulline ratios were observed suggesting that arginase activity was not significantly affected.

We also investigated plasma levels of monomethylarginine (MNMA), asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA), which all may inhibit NOS-dependent NO generation [15]. However, no significant changes in the plasma or dialysate concentrations were observed during the PD sessions (Fig. 5, panel A and B) suggesting that they were not responsible for the reduction in NO signaling. We did however, observe a tendency for a decreased plasma arginine-to-ADMA ratio after PD with standard glucose-based solution (39.0 ± 3.9 vs. 46.9 ± 3.7; P = 0.08). As expected, the concentrations of arginine, citrulline and ornithine increased in the dialysate when using standard glucose-based PD solution, indicating removal during PD (Fig. 5, panel B).

3.3. Single 4-h dwell using an amino-acid-containing PD solution

3.3.1. Markers in plasma and dialysate

Despite normal levels of circulating L-arginine, several studies have shown beneficial effects of supplementation with this amino acid (i.e., the L-arginine paradox) [16]. We recently demonstrated that L-arginine treatment restored NO bioavailability and cGMP signaling in a model of chronic kidney disease [17,18]. In four patients we conducted a single 4-h dwell using an amino-acid-containing PD solution after finishing their prescribed standard PD. The concentration of L-arginine in the amino-acid-containing PD solution was 1.071 g/L. Interestingly we observed no further reduction in plasma nitrate, nitrite (Fig. 6, panel A and B) or cGMP (Fig. 6, panel E) when using the amino-acid containing PD solution. Indeed, the plasma levels of nitrate, nitrite and cGMP tended to increase (P = 0.08, P = 0.07, P = 0.07, respectively) in spite of the significantly increased concentrations of both nitrate and nitrite in the dialysate (Fig. 6, panel C and D). In contrast to what was observed with glucose-based PD solution, plasma arginine-to-ADMA ratio was increased after PD with amino-acid-containing PD solution (57.6 ± 8.8 vs 47.0 ± 6.2; P = 0.03).

Similar to that observed with standard glucose-based PD solution, both citrulline and ornithine levels increased in the dialysate of the amino-acid-containing PD solution. However the level of arginine in the dialysate was reduced suggesting absorption of dialysate arginine. Consistent with this we observed an increase in the plasma levels of arginine during the 4-h dwell using amino-acid-containing PD solution (Fig. 7, panel A and B).

4. Discussion

To the best of our knowledge no previous studies have explored the link between PD-induced reduction of NO bioavailability and
the occurrence of cerebral ischemia in infants. Our results, based on multiple SBP readings, serial neuroimaging, and on repeated measurements of NO markers and signaling in infants on chronic PD indicate that cerebral ischemia is the consequence of a sequence of events whereby persistently low SBP coupled with a reduction in NO bioavailability during the PD session, results in recurrent cerebral hypoperfusion episodes.

Since the autoregulation of CBF is defined as the intrinsic tendency of the brain to maintain a constant blood flow despite variations in perfusion pressure, SBP may play a key role in the maintenance of a safe operating pressure [3,19]. A recent review highlighted the clinical relevance of low SBP in infants on chronic PD [20]. In this review, Vidal and Schaefer acknowledge that low SBP is largely an unrecognized and salient risk factor for ischemic morbidities in this patient population. Moreover, previous studies have shown that low SBP in adult patients on chronic PD is associated with an increased mortality risk [21]. It should be noted that the normal physiologic range for blood pressure, defined by the presence of normal organ blood flow, is unknown in the infants [22]. As a matter of fact, the decision to treat hypotension in the newborn is based on statistically defined age-dependent normative blood pressure values and physician’s believes rather than on data bearing physiologic references. Although few data in infants are available on the relation between SBP and blood flow [23], it is generally considered that early and effective treatment of hypotension can stabilize cardiovascular status and increase the chance of improved neurological outcome and survival in sick preterm and term infants [24]. In children, PD-induced hypotension has been associated with devastating outcomes, such as anterior ischemic optic neuropathy, which is probably linked to hypoperfusion of the posterior ciliary arteries [25]. Of note, in our patients we have not defined the lowest limit of acceptable SBP. In this respect, it has recently been recommended that a threshold of SBP below the 5th percentile for age to define hypotension in infants on chronic PD be applied [20]. However, our previous observations [8] in combination with the current results suggest that higher SBP levels, at least a target of the 50th percentile for age may be a more appropriate threshold. This might result in an increase in perfusion pressure.

Fig. 6. Nitrate, nitrite and cGMP in plasma and dialysate. Analysis of nitrate and nitrite content in plasma (Panel A and B) and dialysate (Panel C and D), both at the beginning (before) and end (after) of each daily PD session with standard glucose-based PD solution (Glu-based PD), as well as after a single 4-h dwell using an amino-acid-containing PD solution (AA-cont. PD), Panel A, B, C, and D, respectively. Changes of plasma cGMP are shown in Panel E. Values are presented as mean ± SEM.
and consequently in an improvement of CBF.

If the autoregulatory property of the cerebral vessels is intact, a reduction in SBP leads to dilatation of the cerebral resistance vessels, while increased pressure constricts the vascular bed to preserve adequate blood flow [19]. Several vasoactive compounds including endothelium-derived NO are known to modulate this autoregulatory property [3,6]. In our study, PD was consistently associated with a lowering of plasma nitrate and nitrite levels and a reduction in NO signaling as indicated by reduced cGMP levels. This may be explained by either a reduced activity of endogenous NOS, or, more likely, due to the abrogation of the alternative nitrate-nitrite-NO pathway [26,27]. It is worth noting that the reduction of plasma nitrate and nitrite levels was almost systematically observed during the investigated PD sessions, whereas no major change in plasma L-arginine was observed. The magnitude of the change in plasma nitrate and nitrite after PD are in the same range as those shown to alter vascular function in adults [28,29]. The fact that nitrate-mediated increases in blood flow predominantly targets areas of tissue hypoxia [30–32], and protects against experimentally-induced ischemia-reperfusion injuries [33–35], support our hypothesis that decreased plasma nitrate and nitrite levels may compromise cerebral perfusion in these areas where it is needed the most. We describe the occurrence of cerebral ischemia during chronic PD with respect to repeated SBP measurements and changes in mean nitrite and nitrate levels but not to fluctuations in cerebral perfusion pressure. Consequently, our hypothesis requires additional studies on the dynamics of cerebral autoregulation that will provide the most physiological indication of disruption in CBF regulation in this high-risk patient population.

Lowering of NO bioavailability or signaling is generally associated with elevated blood pressure and increased cardiovascular risk [27]. However, we do not believe that removal of nitrate and nitrite during the PD session, which may compromise NO signaling, was the cause of the persistently low SBP observed in the infants on chronic PD. In contrast, we propose that a sensitized nitrate-nitrite-NO pathway may actually preserve cerebral perfusion in patients with sustained SBP in the lower range of the normal age- and sex-related reference values. Indeed, our findings indicate that removal of both nitrate and nitrite from plasma and impaired NO signaling during each PD session, might predispose to episodes of transient cerebral vasoconstriction and consequently to ischemic event(s) in infants with low SBP. However, these changes in circulating nitrate and nitrite were not found to jeopardize CBF in children with normal blood pressure. Our findings may lead not only to the development of new treatment strategies to reduce neurological morbidities, but also improve our current understanding of the pathogenesis of cerebral ischemia in infants on chronic PD.

4.1. Future perspectives

Regarding the potential therapeutic value of inorganic nitrate and nitrite, intravenous infusion of these anions may reduce blood pressure further, which is not desirable in this vulnerable group of patients who already display low blood pressure. Instead we propose that supplementation with nitrate and nitrite in the PD solution might be a safe approach to prevent normal losses of these anions during PD. The driving force of solute exchange across the peritoneal membrane is represented by diffusion that is mainly affected by the concentration gradient of the solute between blood and dialysate, its molecular weight, the effective surface area, and permeability of the peritoneal membrane [36]. Hence, it seems reasonable to presume that the infusion of dialysate containing nitrate and nitrite, at concentrations comparable to those found in plasma, can be a safe approach to prevent excessive loss of these inorganic anions during PD. Further clinical studies are clearly necessary to determine the feasibility and the therapeutic value of such an approach.

Author disclosure statement

All the authors, apart from Lundberg J.O. and Weitzberg E., declared no competing interests. Lundberg J.O. and Weitzberg E. are co-inventors on patent applications related to the therapeutic use of inorganic nitrate. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' contribution

M.C. and R.T.K. conceptualized and designed the study and drafted the manuscript. K.W., C.C., A.S., S.H., S.W., Z.B., L.S., P.B. A.C., C.E.W., E.W. and J.O.L. acquired data and/or played an important role in interpreting the results. All the authors critically reviewed and approved the final version of the manuscript. M.C. and R.T.K are the guarantors of the paper.

Acknowledgements

We thank Carina Nihlén and Annika Olsson (Dept. of Physiology and Pharmacology, Karolinska Institutet) for excellent technical assistance throughout the study.
contribution. This work was supported by grants from the Swedish Heart-Lung Foundation (20140448 & 20140469), Swedish Research Council (521-2011-2639), CERIC Linneaus Grant, NovoNordisk Foundation Grant NNF15CC018346, Stockholm City Council(ALF), and by Karolinska Institutet Funding. These funding sources played no role in the study design, collection, analysis, and interpretation of data, the writing of the manuscript, and the decision to submit the manuscript for publication.

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