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

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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 ($-17 \pm 3\%$, $P < 0.05$) and nitrite ($-34 \pm 4\%$, $P < 0.05$) 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 ($-59.4 \pm 15\%$, $P < 0.05$). 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.

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

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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 homesostasis 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 neuroimagings were 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 gravidity-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/m^2 (range 555–702 ml/m^2) 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 neuroimagings, 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 solutions 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