A randomised, double-blind, placebo-controlled trial of the effect of theophylline in prevention of vasomotor nephropathy in very preterm neonates with respiratory distress syndrome

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Keywords: theophylline, vasomotor nephropathy, renal dysfunction, respiratory distress syndrome

Abbreviations:
RDS = respiratory distress syndrome
VLBW = very low birth weight
ELBW = extremely low birth weight
NICU = neonatal intensive care units
ARF = acute renal failure
GFR = glomerular filtration rate
nCPAP = nasal continuous positive airway pressure
SIMV = Synchronized Intermittent Mandatory Ventilation
HFO = High Frequency Oscillation
FiO₂ = Fractional inspired Oxygen
Abstract

Background: Vasomotor nephropathy is a frequently observed renal dysfunction in very preterm neonates.

Objective: To determine whether theophylline could prevent vasomotor nephropathy in very preterm infants with respiratory distress syndrome (RDS).

Methods: Randomized double-blind, placebo-controlled trial of 50 preterm infants with gestational age ≤ 32 weeks needing assisted ventilation. Infants received an intravenous dose of theophylline (1 mg/Kg) or placebo for three days. Twenty-four-hours urine volume was measured daily. On day 2, 5 and 11, blood samples and 12-hours urine collection were analysed for electrolytes, creatinine, urea.

Results: On day 1 urine output was significantly higher in theophylline (2.4±0.9 ml/Kg/h) than in placebo group (1.6±1.0 ml/Kg/h; p=0.023); the incidence of oligoanuria was significantly lower in treated (4.7%) than in placebo group (33%). Twenty-four hours after the first administration of theophylline/placebo, serum creatinine was significantly lower in theophylline (0.76±0.23 mg/dl) than in placebo group (1.0±0.41 mg/dl; p=0.025). On day 5 an increase of serum creatinine was observed in both groups. On day 11 a significant reduction of serum creatinine was observed, as compared to day 5, without difference between the two groups.

Conclusions: Our results suggest that in very preterm infants with RDS early theophylline administration improves renal function during the two first days of life.
Introduction

The premature infant is born with a very low glomerular filtration rate (GFR), that is under control by a delicate balance of intrarenal vasoconstrictor and vasodilator factors, mainly angiotensin II and prostaglandins, respectively. Disorders of vasoactive mediators can further reduce GFR and cause acute renal failure (ARF) or vasomotor nephropathy (VMNP), a renal dysfunction due to reduced renal perfusion. [1][2]

ARF has been estimated to affect 8-26% of neonates admitted to neonatal intensive care units (NICU), and may result in persistent glomerular and/or tubular dysfunction in 35-40% of cases.[3][4][5][6] Perinatal hypoxemia or asphyxia, in the course of a severe respiratory distress syndrome (RDS), is one of the most frequent conditions of neonatal ARF due to adenosine activation.[1] [5] [7][8] Experimental data have demonstrated that theophylline, a xanthine derivative with adenosine antagonistic properties, is able to reverse the intrarenal vasoconstriction observed during hypoxemia.[9][10][11][12][13]

The aim of our study was to determine whether theophylline could prevent VMNP in very preterm infants with RDS, in a randomised double-blind, placebo-controlled trial.

Methods

Inborn preterm neonates, with ≤ 32 weeks gestational age, who developed RDS within 6 hours from birth and needed mechanical ventilation or nasal continuous positive airway pressure (nCPAP) were included in the study.

Exclusion criteria were kidneys and/or urinary tract congenital abnormalities, congenital hearth defects, prenatal exposure to ACE-inhibitors or non-steroidal anti-inflammatory drugs (NSAID), and chromosomal disorders or multiple malformations.

The study protocol was approved by the Hospital Ethical Committee. Babies were enrolled after written informed consent was obtained from parents.

Neonates were randomised by computer-generated numbers to receive a daily intravenous dose of theophylline (1 mg/Kg) (Aminomal, Malesci) or an equal volume of placebo (5% dextrose in water) for three consecutive days. The first dose of drug/placebo was given soon after inclusion criteria were verified. The Neonatal Intensive Care Unit (NICU) medical and nursing staff was blinded to the patient assignment. Theophylline or placebo were prepared by a physician from the Neonatology section, not involved in patients care, using syringes with identical external appearance, following the randomisation allocation table.

The rate of intravenous fluids were determined by the attending staff according to the protocol adopted in our NICU (total fluid input day 1: 70 ml/Kg; day 2: 90 ml/Kg; day 3: 110 ml/Kg; day 4: 120 ml/Kg; day 5: 140 ml/Kg; day 6: 150 ml/Kg; day 7: 150 ml/Kg). On day 1 babies received an intravenous infusion of 10% dextrose in water; parenteral nutrition was begun on the second day with the same amounts of lipid and amino acid solutions in both groups. Neonates were commenced on enteral feeding as soon as their clinical condition permitted; the volume of enteral feeding was included in the fluid volume received. No sodium was added to maintenance intravenous fluids during the first 24 hours after birth. Subsequently, sodium intake was adjusted to maintain serum sodium level between 135 and 145 mEq/l. Antibiotics, inotropics (dopamine, dobutamine), diuretics (furosemide), analeptics (caffeine), NSAID (ibuprofen, indomethacin) and...
surfactant were prescribed as indicated by the clinical status of each patient. All fluid volumes of administered infusions and medications were carefully recorded by nursing staff. Body weight was determined at birth and every 24 hours; blood pressure was measured hourly by the oscillometric method; cardio-respiratory activity, oxygen saturation and transcutaneous PaO₂ and PaCO₂ were recorded continuously.

Urine was collected daily on open nappies after spontaneous voiding or application of suprapubic pressure.[14] The 24-hour urine volume was measured starting from birth by weighing the diapers every two hours.

After the first 24 hours of life (day 2) blood samples were drawn and analysed for electrolytes, creatinine, urea. Twelve hours urine collection was obtained using an external device attached to the genitalia; neonates were observed for spontaneous voiding and the collection interval was initiated and ended immediately after the voiding; bladder emptying was confirmed at this time by applying suprapubic pressure. The volume of all urine collected during this timed interval was measured and an aliquot was analyzed for electrolytes. Oliguria was defined as an urine output < 1 ml/Kg/h for at least 24 hours. The same measurements were performed in blood and urine samples on days 5 and 11. On day 5 serum theophylline level was measured. On days 5 and 11 the urine concentration of β2-microglobulin in the 12h-urine samples was measured as index of tubular function; normal upper limit was 4.0 mg/L (mean + two standard deviation in healthy infants).[15]

Blood urea was determined by the urease/GLDH method, creatinine by Jaffè method; serum theophylline levels were determined by Fluorescent Polarisation Immuno Assay (FPIA) and β2-microglobulin by nephelometric immunoassay.[16][17][18]

The creatinine clearance was estimated using Schwartz’s formula for preterm neonates: creatinine clearance (ml/min/1.73 m²)=0.33x length (cm)/plasma creatinine (mg/dl).[19][20][21]

Incidence of the following complications were considered: 1) Patent ductus arteriosus (PDA) 2) Intraventricular haemorrhage 3) Periventricular leukomalacia 4) Retinopathy of prematurity 5) Bronchopulmonary dysplasia 6) Necrotising enterocolitis.

Statistical analysis was performed by the two way repeated measures analysis of variance (ANOVA), followed by the Newman-Keuls “post hoc” test, to evaluate differences of renal function between the two groups at different times. The baseline clinical data of the two groups were analysed by unpaired two tailed Student t test, or by χ² test with Yates correction, as required. The projected number of needed subjects was calculated by selecting a power of 0.9 and a two-tailed alpha of 0.05. Sample size was estimated to be 20 infants for each study group to demonstrate a 20% difference of urine output, serum creatinine, GFR or blood urea between the two groups. To compensate for non assessable patients, we planned to enrol 50 infants. A p value > 0.05 was considered not significant. All computations were performed using a commercial statistical package (STATISTICA for Windows, StatSoft, Inc, Tulsa, OK, USA).

Results

During a 12-months period, 54 consecutive preterm neonates met the entry criteria, four sets of parents did not give informed written consent, 50 babies were randomized, three neonates died during the first 48 hours leaving 47 infants who completed the study (Fig. 1).
Patients in the two groups had similar characteristics. Twenty-five neonates (16 males, 9 females) were randomised to receive placebo (group P). Twenty-five babies (13 males, 12 females) were assigned to the theophylline group (group T).

There were no significant differences of mean gestational age, birth weight, or number of babies submitted to mechanical ventilation, Synchronized Intermittent Mandatory Ventilation (SIMV) or High Frequency Oscillation (HFO) or nCPAP, maximum Fractional inspired Oxygen (FiO₂) requirement and hourly mean arterial blood pressure between the two groups during the first five days (Table 1).

**Table 1.** Clinical characteristics of infants on the first day of life and drug therapy administered during the study. The value are expressed as mean±standard deviation or number of patients.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=25)</th>
<th>Theophylline (n=25)</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>28.7±1.6</td>
<td>28.7±2.0</td>
<td>N.S.*</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1157±354</td>
<td>1192±378</td>
<td>N.S.*</td>
</tr>
<tr>
<td>SIMV</td>
<td>15</td>
<td>14</td>
<td>N.S.*</td>
</tr>
<tr>
<td>HFO</td>
<td>3</td>
<td>4</td>
<td>N.S.*</td>
</tr>
<tr>
<td>NCPAP</td>
<td>7</td>
<td>7</td>
<td>N.S.*</td>
</tr>
<tr>
<td>FiO₂ max</td>
<td>0.39±0.12</td>
<td>0.44±0.18</td>
<td>N.S.**</td>
</tr>
<tr>
<td>Mean Arterial pressure</td>
<td>33.0±7.0</td>
<td>35.4±6.0</td>
<td>N.S.**</td>
</tr>
<tr>
<td>Furosemide</td>
<td>12</td>
<td>8</td>
<td>N.S.*</td>
</tr>
<tr>
<td>Dopamine</td>
<td>21</td>
<td>21</td>
<td>N.S.*</td>
</tr>
<tr>
<td>Dobutamine</td>
<td>12</td>
<td>9</td>
<td>N.S.*</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>4</td>
<td>6</td>
<td>N.S.*</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>1</td>
<td>0</td>
<td>N.S.*</td>
</tr>
<tr>
<td>Caffeine</td>
<td>9</td>
<td>8</td>
<td>N.S.*</td>
</tr>
</tbody>
</table>

* values calculated from $χ^2$ analysis
** Student $t$ test

No differences of diuretics, inotropic drugs, NSAID for PDA or caffeine use between the two groups were found (Table 1).

During the first 24 hours of life (day 1) urine output (ml/Kg/h) was significantly higher in the theophylline group (2.4±0.9 ml/Kg/h) as compared to the placebo group (1.6±1 ml/Kg/h; p=0.023) (Fig. 2). The incidence of oligoanuria (urine output < 1ml/kg/h) was significantly lower in the theophylline group after the first study day (1/21 neonates, 4.7%, vs 8/24 neonates, 33%; p=0.017).

No significant difference of urine output was observed over the next ten study days between the two groups (Fig. 2).

On day two, 24 hours after the first administration of theophylline/placebo, neonates in the theophylline group had significantly lower serum creatinine level (67.3±20.4 µmol/l) than the placebo group (89.8±36.3 µmol/l; p=0.025) (Fig. 3). The creatinine clearance was significantly higher in the theophylline group (19.0±9.6 ml/min/1.73m²) than in the placebo group (13.5±3.6 ml/min/1.73/m²).
Similar results were found in the two subgroups of neonates with severe RDS who required tracheal intubation and mechanical ventilation (17 infants in each group): group T creatinine 64.6±21.2 µmol/l, group P creatinine 92.0±40.7 µmol/l, p=0.035; group T creatinine clearance 19.9±10.7 ml/min/1.73m², group P creatinine clearance 13.7±3.7 ml/min/1.73m², p=0.037.

At five days of life, a reduction of renal function, evaluated by serum creatinine and creatinine clearance, was observed in both groups as compared to the second day; mean serum creatinine in the group T was lower (95.1±30.6 µmol/l) compared with the group P (107.4±36.1 µmol/l) and creatinine clearance in the group T was higher (13.1±4.4 ml/min/1.73m²) compared with the group P (11.1±3.6 ml/min/1.73m²) although these differences were not significant. At eleven days a significantly improvement of the renal function, as compared with the fifth day, was observed in both groups, with no differences of serum creatinine or creatinine clearance values.

Blood urea in group T was lower compared with the group P on day 2 (group T 4.9±1.9 mmol/l, group P 5.9±2.6mmol/l), although the difference was not significant (p=0.052). On day 5 and 11 serum urea level, like creatinine, was lower in the neonates receiving theophylline although not significantly.

Mean serum sodium and potassium levels were within normal limits on day 2, 5 and 11, without significant differences between the two groups. The incidence of hyponatremia (Na < 130 mEq/l) and hyperkalemia (K > 6.5 mEq/l) was very low in both groups (group T: hyponatremia 2 infants, hyperkalemia 3 infants; group P: hyponatremia 2 infants, hyperkalemia 2 infants). Sodium intake (mEq/Kg), sodium excretion (mEq/24h) and sodium balance was similar in the two study groups on day 2, 5 and 11.

Urinary β2-microglobulin concentrations on the fifth and eleventh days of the study resulted higher than normal in five and in four infants of group T and P, respectively. At these times no difference was found of the mean urinary β2-microglobulin values between groups (group T day 5: 4.0±4.6 mg/l, day 11: 3.7±4.5 mg/l; group P day 5: 4.0±4.5 mg/l, day 11: 2.5±2.8 mg/l; p=NS).

The theophylline group achieved an average serum theophylline level of 2.7±2.3 µg/dl on day 5, while serum level was not measurable in the placebo group.

No significant differences in incidence of complication (Table 2) were found. One baby of group T died after the study.
Table 2. Main complications in the two infant groups. Values are expressed as mean±standard deviation or number of patients.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=24)</th>
<th>Theophylline (n=23)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of stay (days)</td>
<td>62.3±25.9</td>
<td>62.7±29.9</td>
<td>N.S.**</td>
</tr>
<tr>
<td>Patent ductus arteriosus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraventricular haemorrhage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I grade</td>
<td>4</td>
<td>2</td>
<td>N.S.*</td>
</tr>
<tr>
<td>II grade</td>
<td>1</td>
<td>0</td>
<td>N.S.*</td>
</tr>
<tr>
<td>III grade</td>
<td>1</td>
<td>1</td>
<td>N.S.*</td>
</tr>
<tr>
<td>Periventricular leukomalacia</td>
<td>2</td>
<td>2</td>
<td>N.S.*</td>
</tr>
<tr>
<td>Retinopathy of prematurity</td>
<td>2</td>
<td>2</td>
<td>N.S.*</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia</td>
<td>4</td>
<td>1</td>
<td>N.S.*</td>
</tr>
<tr>
<td>Necrotising enterocolitis</td>
<td>1</td>
<td>0</td>
<td>N.S.*</td>
</tr>
</tbody>
</table>

* values calculated from $\chi^2$ analysis  
** Student $t$ test

Discussion

Our results show that in very preterm infants, low-dose theophylline administration at the beginning of RDS improves renal function in the first two days of life. Indeed, treated infants showed a better urine output, with a significant reduction of oliguria, and serum creatinine level significantly lower and endogenous creatinine clearance significantly higher than the placebo group. Creatinine clearance was estimated by Schwartz equation. Although this adds limited additional knowledge to serum creatinine concentration alone, the Schwartz equation, because of the difficult to obtain an accurate timed urine collection, remains the most widely used marker of creatinine clearance in the neonatal age. [19][20][21]

The approach to acute renal failure today is mainly oriented to the prevention.[1] It is therefore possible to attempt an early modulation of the mediators responsible for kidney hypoperfusion, glomerular filtration rate reduction, and subsequent cellular injury.

Several experimental evidences seem to confirm the key role of adenosine in the pathogenesis of renal failure secondary to hypoxemia.[8][22][23][24][25] Theophylline may antagonize renal endogenous adenosine, increased after renal hypoxia or ischemia.[26][27] Some authors have described an acute effect of theophylline on urine output when given to preterm babies for apnea of prematurity.[28][29][30] A significant increase of urine output and creatinine clearance was reported in five out of six neonates with RDS, 12 hours after the administration of a single dose of 1 mg/Kg theophylline.[13] In a prospective randomised study on term asphyxiated neonates the prophylactic early theophylline given at birth as a single 8 mg/Kg dose, showed a protective effect on renal function, with significant increase of urine output and creatinine clearance from the second to fifth day of life, and reduction of urinary β2-microglobulin.[14]
In our study the theophylline preventive effect on renal function did not persist after the first two days. It is well known that preterm infants show a short-duration initial increase in plasma creatinine concentration because of creatinine tubular reabsorption.[31][32][33][34] Therefore, a renal failure should be hypothesized when a further increase or a lack of reduction of creatinine serum level is observed during the first week of life.

The preterm kidney is very vulnerable to multiple factor that can modify its hemodynamics and determine renal failure.[35][36] In addition to hypoxemia, several pathological and iatrogenic conditions during the neonatal period can modify renal hemodynamics by the activation of different vasoactive factors further than adenosine, as the renin-angiotensin system, endothelin, atrial natriuretic peptide, prostaglandins and thromboxane.[1][2]

The theophylline protective effect on renal function noticed at the beginning of respiratory distress in our preterm neonates could be induced by inhibition of renal endogenous adenosine, which increases during hypoxemia. Other pathogenetic factors, as sepsis, mechanical ventilation and nephrotoxic drugs, that activate mediators not affected by theophylline may explain the worsening of renal function after the first few days.

As the majority of the patients included in the study, both in the control and in the treatment group, received dopamine treatment, we may hypothesize that the observed effects of theophylline are applicable principally to patients on dopamine.

Further studies are necessary to verify whether the short term improvement of renal function induced by low dose theophylline may prove useful to improve the prognosis of very preterm infants with RDS. Indeed, even a moderate improvement in renal function in critical situations is appreciable and may ease the whole management of many patients.
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Competing interest statement
On behalf of all authors I disclaim any conflict of interest. The authors have not received any payments for conducting or publicizing the above study.

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What is already known on this topic
a) Vasomotor nephropathy is a frequently observed renal dysfunction in very preterm neonates
b) Theophylline can reverse the intrarenal vasoconstriction observed during hypoxemia

What this study adds
a) In very preterm infants with RDS early theophylline administration improves renal function during the two first days of life
b) Treated infants showed a better urine output, significantly lower serum creatinine level and significantly higher endogenous creatinine clearance than the placebo group
c) A moderate improvement in renal function in critical situations is appreciable and may ease the whole management of many patients

Figure legends

Fig 1
Trial profile

Fig 2
Urine output (mean ± standard deviation) during the study. On day 1 the neonates in group T had significantly higher urine output than neonates in group P (*p=0.023).

Fig 3
Serum creatinine (mean ± standard deviation) on day 2, 5, 11 and at discharge. On day 2 the neonates in group T had significantly lower serum creatinine than neonates in group P(*p=0.0025).
References


54 ELIGIBLE NEWBORNS

50 RANDOMLY ALLOCATED

25 ASSIGNED PLACEBO
- 1 DIED
- 24 EVALUABLE AND ASSESSED

25 ASSIGNED THEOPHYLLINE
- 2 DIED
- 23 EVALUABLE AND ASSESSED

4 REFUSED