Bumetanide for the treatment of seizures in newborn babies with hypoxic ischaemic encephalopathy (NEMO): an open-label, dose finding, and feasibility phase 1/2 trial

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Summary

Background **Preclinical data suggest that the loop-diuretic bumetanide might be an effective treatment for neonatal seizures. We aimed to assess dose and feasibility of intravenous bumetanide as an add-on to phenobarbital for treatment of neonatal seizures.**

Methods **In this open-label, dose finding, and feasibility phase 1/2 trial, we recruited full-term infants younger than 48 h who had hypoxic ischaemic encephalopathy and electrographic seizures not responding to a loading-dose of phenobarbital from eight neonatal intensive care units across Europe. Newborn babies were allocated to receive an additional dose of phenobarbital and one of four bumetanide dose levels by use of a bivariate Bayesian sequential dose-escalation design to assess safety and efficacy. We assessed adverse events, pharmacokinetics, and seizure burden during 48 h continuous electroencephalogram (EEG) monitoring. The primary efficacy endpoint was a reduction in electrographic seizure burden of more than 80% without the need for rescue antiepileptic drugs in more than 50% of infants. The trial is registered with ClinicalTrials.gov, number NCT01434225.**

Findings **Between Sept 1, 2011, and Sept 28, 2013, we screened 30 infants who had electrographic seizures due to hypoxic ischaemic encephalopathy. 14 of these infants (10 boys) were included in the study (dose allocation: 0·05 mg/kg, n=4; 0·1 mg/kg, n=3; 0·2 mg/kg, n=6; 0·3 mg/kg, n=1). All babies received at least one dose of bumetanide with the second dose of phenobarbital; three were withdrawn for reasons unrelated to bumetanide, and one because of dehydration. All but one infant also received aminoglycosides. Five infants met EEG criteria for seizure reduction (one on 0·05 mg/kg, one on 0·1 mg/kg and three on 0·2 mg/kg), and only two did not need rescue antiepileptic drugs (ie, met rescue criteria; one on 0·05 mg/kg and one on 0·3 mg/kg). We recorded no short-term dose-limiting toxic effects, but three of 11 surviving infants had hearing impairment confirmed on auditory testing between 17 and 108 days of age. The most common non-serious adverse reactions were moderate dehydration in one, mild hypotension in seven, and mild to moderate electrolyte disturbances in 12 infants. The trial was stopped early because of serious adverse reactions and limited evidence for seizure reduction.**

Interpretation **Our findings suggest that bumetanide as an add-on to phenobarbital does not improve seizure control in newborn infants who have hypoxic ischaemic encephalopathy and might increase the risk of hearing loss, highlighting the risks associated with the off-label use of drugs in newborn infants before safety assessment in controlled trials.**

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Introduction

Acute seizures are the most common neurological emergency in newborn babies, arising in roughly three per 1000 term livebirths, most commonly due to hypoxic ischaemic encephalopathy. Neonatal seizures are a major challenge for clinicians because of inconspicuous clinical presentation, variable electroclinical correlation, and poor response to antiepileptic drugs.[1–3]

In the neonatal period, increased susceptibility to seizures and poor response to antiepileptic drugs might be related to age-dependent differences in intracellular chloride concentrations caused by high expression of the sodium/potassium/chloride co-transporter isoform 1 (NKCC1). This high expression results in a depolarising (excitatory) response of GABA receptors in immature neurons by contrast with a hyperpolarisation (inhibitory) response in adult neurons (appendix p 6).[4,5] Data from experimental studies have suggested that modification of the GABA receptor response by blocking NKCC1 with bumetanide might be effective against neonatal seizures.[5–7] However, only one study[6] in a hypoxia-induced seizure model has presented convincing in-vivo evidence that bumetanide together with phenobarbital significantly reduced seizure burden, whereas neither bumetanide nor phenobarbital alone were effective.

Bumetanide is a loop diuretic widely used in newborn babies.[8,9] Pharmacokinetic studies in children and infants report a good safety profile with a half-life of roughly

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See Online for appendix

6 h.[10] Previously described adverse reactions are linked to the diuretic effect of bumetanide including fluid loss, dehydration, electrolyte disturbances, and hyperglycaemia.[8–10] In keeping with all loop diuretics, a theoretical risk of hearing loss exists, although this risk is not usually deemed high with bumetanide.[11]

Data from in-vivo studies in rodent models of epilepsy suggested an optimum dose of 0·1–0·3 mg/kg intraperitoneal bumetanide for seizure reduction,[5,6] which is higher than the range of doses prescribed for diuresis (0·05–0·1 mg/kg).[8,9] The diuretic effect is maximum at doses of 0·04 mg/kg, hence high doses of bumetanide are not associated with increased diuresis or electrolyte imbalance.[12] The optimum anticonvulsant dose in human beings and safety profile at higher doses are unknown. Additionally, the ability of bumetanide to penetrate the blood–brain barrier is unclear.

Nevertheless, data suggest that bumetanide might be an effective and safe add-on treatment for difficult-to-treat neonatal seizures, and in 2008 the European Medicines Agency (EMA) included bumetanide for neonatal seizures on their priority list, which aims to direct EU funding into research on drugs that target disorders in children for which treatments are not available. Specifically, the EMA encouraged the assessment of the efficacy of bumetanide as an add-on to phenobarbital for neonatal seizures. Ultimately, only a proof-of-concept clinical trial in newborn babies can establish whether preclinical data translate to clinical practice. We did a phase 1/2 dose finding and feasibility study to assess the safety and optimum dose of bumetanide for the treatment of refractory neonatal seizures after a loading dose of phenobarbital, assessing four dose levels of bumetanide.

For the **EMA priority list** see http://www.prescrire.org/docus/EMEA22698308en.pdf

Methods

Study design and participants

In this open-label, dose finding, and feasibility trial, we recruited babies from eight neonatal intensive care units in five countries in Europe (UK, Ireland, the Netherlands, Sweden, and Finland). The trial protocol, which can be requested via the study website, was approved by the regulatory authority of each participating country, and by the national or institutional ethical review board of every centre. Because of the acute and time-sensitive nature of early neonatal seizure treatment, a continuous consent process[13] was followed. Infants with moderate to severe hypoxic ischaemic encephalopathy were identified as soon as possible after birth and electroencephalogram (EEG) monitoring was started. Parents were informed at this point about the study. If seizures were confirmed on EEG, written informed consent was obtained. Infants started the study protocol if seizures recurred after initial treatment, and if all inclusion criteria were met. Parents were updated, and consent reaffirmed, at least once a day by the study team during the treatment period.

For the **study website** see http://www.nemo-europe.com/

Clinical inclusion criteria included: gestational age of 37–43 weeks and postnatal age younger than 48 h; evidence of perinatal asphyxia (defined as a 5 min Apgar score ≤5, severe acidosis with umbilical cord or first arterial blood sample pH ≤7·10, a base deficit of ≥16 mmol/L, or continued need for resuscitation 10 min after birth); clinically evolving encephalopathy; and electrographic seizures not responding to 20 mg/kg of phenobarbital (≥3 min cumulative seizures, or two or more seizures of >30 s during 2 h). Exclusion criteria included: administration of other diuretics or anticonvulsants (other than midazolam for intubation); major congenital abnormalities; inborn errors of metabolism; genetic syndromes; and unacceptable abnormalities of electrolytes, total bilirubin, or creatinine (appendix p 2).

Procedures

Bumetanide, provided by Only For Children Pharmaceuticals (O4CP), was given as a clear solution for injection (2 mg/mL in 5 mL vials) in four dose levels (0·05 mg/kg, 0·1 mg/kg, 0·2 mg/kg, and 0·3 mg/kg) four times intravenously via slow infusion at 12 h intervals (maximum of 1·2 mg/kg). Excipients in water were xylitol, disodium hydrogen phosphate dodecahydrate (buffer), and sodium hydrogen phosphate dehydrate (buffer). The initial dose was given with a second single dose of phenobarbital (10 mg/kg; figure 1 and appendix p 7). Baseline investigations were clinical assessment of hypoxic ischaemic encephalopathy grade and routine clinical and laboratory status.

Participants were monitored with continuous video EEG (minimum eight channels) for 48 h after start of the study drug. Two independent neurophysiologists assessed seizure burden masked to patient identity, time of recording (before and after treatment), and dose allocation. Unanimous annotations were taken as definitive seizures; a seizure was judged to have occurred if both neurophysiologists noted a seizure at the same point in the video recording or by a consensus agreement, when the initial annotations diverged. The diagnosis of an electrographic seizure needed the evolution of sudden, repetitive, evolving stereotyped EEG patterns with a definite beginning, middle, and end, lasting longer than 10 s.[3,14] For screening of infants with moderate to severe hypoxic ischaemic encephalopathy, local investigators identified seizures with either amplitude-integrated EEG or the raw multichannel EEG signal; however, after inclusion in the study, monitoring during the treatment phase and subsequent analysis was always with full continuous EEG. The 2 h of EEG recording immediately before administration of the first bumetanide dose was regarded as baseline.

The study protocol allowed administration of rescue drugs at any time if seizure frequency was considered clinically unacceptable by the clinician in charge. Safety was monitored continuously (appendix p 3). The end of the trial was marked by the completion of a hearing

test in the last infant enrolled. Screening for hearing impairment was done as part of the Universal Newborn Hearing Screening, which is now standard practice in all infants admitted to intensive care. Screening was done at, or shortly after discharge from hospital, but within the first month of life, using otoacoustic emissions or automated auditory brainstem response. Confirmatory testing was obtained with automated auditory brainstem response.

Outcomes

In view of previous clinical trials and expert opinion, we assumed that 20% of infants would either respond to the second dose of phenobarbital (10 mg/kg) or spontaneously stop having seizures.[3,15,16] The pre-specified efficacy endpoint was a reduction of 80% or more compared with baseline in electrographic seizure burden (min/h)[15] during hours 3–4 after first bumetanide administration, with no need for rescue antiepileptic drugs within 48 h, and arising in more than 50% of infants.

The primary safety endpoint was defined as the following, within 48 h of the first dose: absence of suspected unexpected serious adverse reactions; serious adverse reactions that were deemed probably related to bumetanide in less than 10% of infants; and less than 10% of infants with adverse reactions related to diuresis (severe hypokalaemia [<2·8 mmol/L] or electrocardiogram changes or both, or severe dehydration [dehydration with hypotension <35 mm Hg needing inotropic support]).

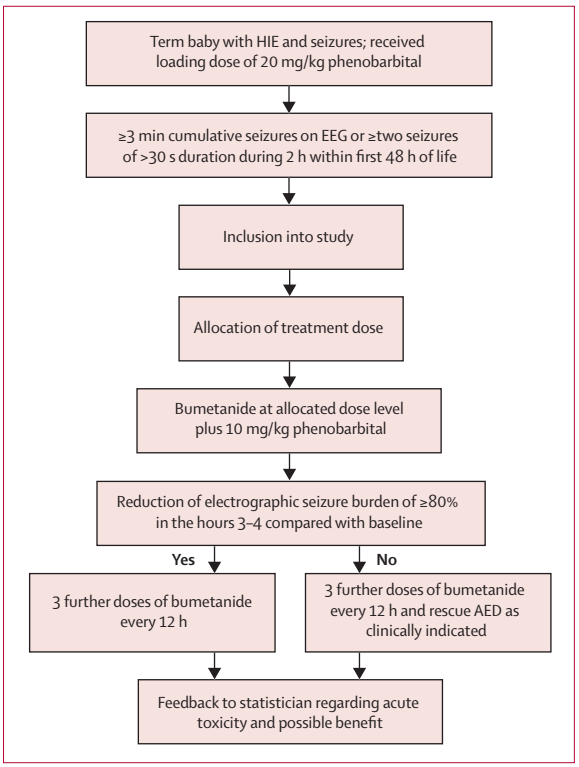

Figure 1: **Procedures for enrolment and dose allocation**
HIE=hypoxic ischaemic encephalopathy. EEG=electroencephalogram. AED=antiepileptic drug.

Statistical analysis

We used a modified bivariate (safety and efficacy) adaptive model to estimate the most successful safe dose[17]—ie, the dose that maximised the probability of efficacy under predefined toxic effect restrictions. Pairs of babies received the same dose regimen, according to assignment tables and as established by the statistician on the basis of the distribution of the a-posteriori probabilities of success for the preceding two patients. We worked out the sample size on the basis of simulation studies of the proposed method.[17] The bivariate continual reassessment method is based on a Bayesian inference in which no sample size calculation is theoretically needed to estimate the safe most successful dose. However, on the basis of simulation studies, the sample size of 24 infants (with four dose levels and cohort size of two) would allow us to estimate the correct dose in more than 80% of cases (appendix p 1).

Fixed standards for the minimum probability of response (set as 50%) and for the maximum probability of non-tolerance (set as 10%) were pre-specified. Analysis was by intention to treat. Stopping rules were pre-specified (appendix p 8). Pharmacokinetic assessment used a population approach. Four blood samples per participant were taken at pre-allocated times in two time schedules. Concentration–time data were analysed with Monolix software (version 4.2). We chose the final pharmacokinetic model on the basis of the value of the objective function and on visual inspection of classical goodness-of-fit plots and visual predictive checks. The area-under-the-curve of bumetanide was estimated with Bayesian statistics. The Bayesian estimation of pharmacokinetic parameters provides an estimated pharmacokinetic parameter with fewer patients than traditional pharmacokinetic methods by using the individual pharmacokinetic data and previous data from other patients in similar populations.[18]

This study is registered at ClinicalTrials.gov, number NCT01434225.

Role of the funding source

The funders had no responsibility for the design of the study, for collection, analysis, and interpretation of data, for writing of the trial report, or in the decision to submit the paper for publication. Data interpretation was the responsibility of the trial steering committee (advised by an independent drug monitoring committee). All authors had full access to study data; the corresponding author had final responsibility for the decision to submit the report for publication.

Results

Between Sept 1, 2011, and Sept 28, 2013, we screened 30 infants with electrographic seizures due to hypoxic ischaemic encephalopathy. Of those, 14 (ten boys)

For the **UK Universal Newborn Hearing Screening programme** see http://hearing.screening.nhs.uk/audiology

	Gestational age (weeks plus additional days at birth)	Sex	Type of delivery	APGAR scores at 1, 5, and 10 min after birth	First blood gas test			Hypoxic ischaemic encephalopathy (Sarnat stage)	Aminoglycosides*	Therapeutic hypothermia (starting time; h after birth)
					Blood gas type	Base excess	First pH			
1	39+5	Boy	Ventouse	1/4/7	Capillary	−16	7·18	2	Tobramycin	Yes (9·5†)
2	42+1	Boy	Ventouse	1/3/6	Capillary	−13	7·26	3	Tobramycin	Yes (3·0)
3	40+3	Boy	Emergency caesarean section	0/1/2	Venous	−19·5	6·83	3	Gentamicin	Yes (5·7)
4	41+0	Boy	Spontaneous vaginal delivery	9/10/10	Arterial	−17	6·97	3	Gentamicin	Yes (5·9)
5	40+1	Boy	Elective caesarean section	0/1/2	Capillary	−18·5	6·91	3	Gentamicin	Yes (5·1)
6	40+0	Boy	Ventouse	1/2/7	Capillary	−5·5	7·15	2	Tobramycin	Yes (12·3†)
7	41+3	Girl	Ventouse	3/6/6	Arterial	−13	7·24	2	Gentamicin	Yes (5·5)
8	39+3	Girl	Elective caesarean section	3/6/6	Arterial	−12·2	6·95	3	Gentamicin	Yes (5·5)
9	41+1	Girl	Spontaneous vaginal delivery	NA	Capillary	NA	6·8	2	Gentamicin	Yes (1·2)
10	37+6	Boy	Spontaneous vaginal delivery	2/2/2	Arterial	−21	6·87	2	Tobramycin	Yes (3·3)
11	41+2	Boy	Spontaneous vaginal delivery	2/6/8	Capillary	−17·3	6·95	2	Gentamicin	No
12	39+6	Boy	Spontaneous vaginal delivery	2/6/7	Arterial	−20·1	6·80	2	Gentamicin	Yes (1·4)
13	40+6	Boy	Ventouse	1/5/7	Arterial	−20·7	6·86	2	Gentamicin	Yes (1·6)
14	41+1	Girl	Ventouse	1/4/4	Arterial	−18·5	6·6	3	None	Yes (2·3)

NA=not available. *One dose 4 mg/kg. †Passive cooling was started within 6 h of birth.

Table 1: **Baseline characteristics**

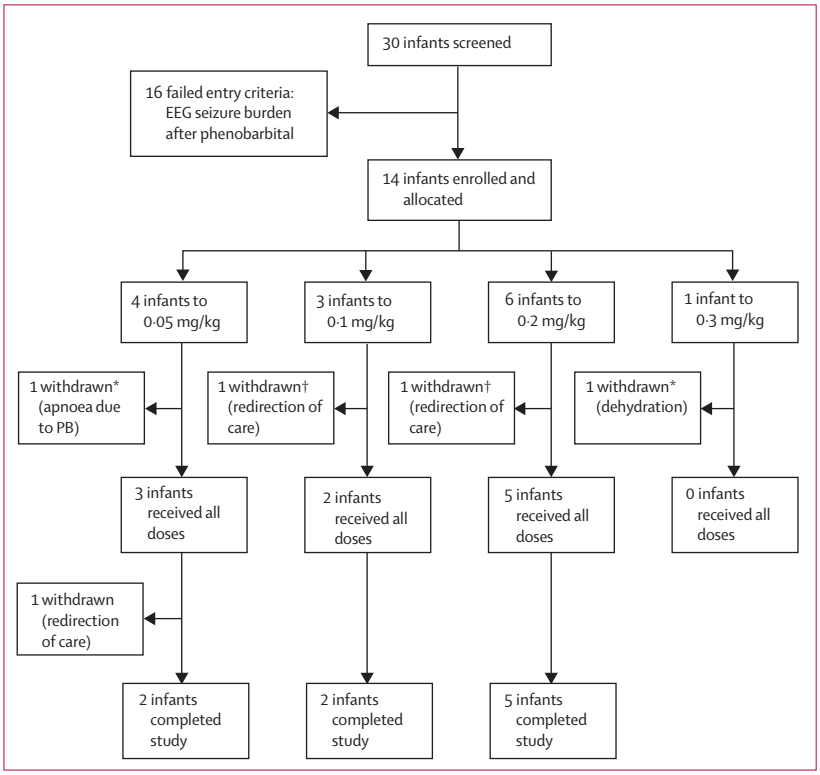

Figure 2: **Trial profile**
Two infants were withdrawn before the end of the treatment phase because of changes in clinical management (redirection of clinical care), one infant was withdrawn because of apnoea after a total dose of 40 mg/kg phenobarbital, and one infant because of dehydration (drug-related adverse reaction). Another infant completed the treatment phase but a clinical decision was made to redirect clinical care before the end of the trial (hearing test); thus, the observation period was shortened. *Received three doses. †Received one dose. EEG=electroencephalogram. PB=further dose of phenobarbital.

continued to have seizures after phenobarbital treatment and were enrolled in the study (table 1). On the basis of clinical assessment, eight had moderate and six had severe hypoxic ischaemic encephalopathy. 13 infants received whole-body therapeutic hypothermia for neonatal encephalopathy, cooling to a targeted temperature of 33°C for 72 h. 13 received aminoglycosides (gentamicin n=9, tobramycin n=4) for 0–9 days (mean 3·6 days).

Four infants were allocated to a dose of 0·05 mg/kg of bumetanide, three to 0·1 mg/kg, six to 0·2 mg/kg, and one to 0·3 mg/kg (figure 2). All infants received at least one dose of bumetanide, and ten infants completed treatment as per protocol. The primary efficacy outcome measure was available for all included infants. Electrographic seizure burden at baseline varied between 0 and 34 min/h. All infants had seizures in the inclusion period (meeting all inclusion criteria) but, because of marked hourly fluctuations of seizure burden and the logistical delays imposed by having to obtain consent and prepare the drug, five infants had no seizures in the baseline period. Five of 14 infants had a greater than 80% reduction of seizure burden after the first dose of bumetanide (table 2). A further two patients (patient numbers 7 and 8) had a more than 50% seizure reduction in the 3–4 h after first bumetanide administration. However, rescue drug was given to 12 infants (within 4 h of the initial administration of bumetanide in five infants), which confounded the subsequent assessment of seizure burden (table 2).

Within the interventional period, six infants received one additional antiepileptic drug, five received two, and one received three. Choices of second line antiepileptic drugs were midazolam (n=8), phenytoin (n=5), further

	Bumetanide dose (mg/kg)	Cumulative dose (mg/kg)	Time from start of cooling to first dose of bumetanide (h)*	Time from initial phenobarbital to first dose of bumetanide (h)*	Safety concern	Hearing tests†	Hearing test results		Amino-glycoside (mmol/L) trough level	Seizure burden (min/h)			Rescue AED	
							Right	Left		Baseline	3–4 h	24 h	4 h	5–48 h
1	0·05	0·2	5·4	5·6	None	Otoacoustic emissions	Normal	Normal	1·00 mmol/L	0·00	0·00	0·02	None	None
2	0·05	0·2	18·5	10·9	Hearing loss	Otoacoustic emissions and automated auditory brainstem	50 dB	Normal	1·10 mmol/L	10·26	13·39	2·82	Midazolam	Midazolam
3	0·05	0·15	24·5	13·1	None	Otoacoustic emissions	Normal	Normal	1·70 mmol/L, 2·60 mmol/L	10·72	0·00‡	1·31‡	None	Phenytoin
4	0·05	0·2	18·2	19·7	Death†	NA§	NA§	NA§	NR	0·17	3·81	0·82	Midazolam	Lidocaine, PB
5	0·1	0·1	6·3	3·3	Death†	NA§	NA§	NA§	NR	0·36	0·00‡	NA	None	Midazolam
6	0·1	0·4	1·4	5·4	None	Otoacoustic emissions	Normal	Normal	1·60 mmol/L	0·00	0·00	0·00	None	Midazolam, PB
7	0·1	0·4	3·4	3·4	None	Otoacoustic emissions	Normal	Normal	6·70 mmol/L	33·98	16·08	NA	Phenytoin	Phenytoin, midazolam
8	0·2	0·2	21·1	10·7	Death†	NA§	NA§	NA§	NR	15·98	8·32	3·80	Midazolam	Lidocaine, midazolam
9	0·2	0·8	25·9	22·7	None	Otoacoustic emissions	Normal	Normal	2·40 mmol/L, 1·60 mmol/L	11·59	0·24‡	0·95‡	None	Phenytoin, midazolam
10	0·2	0·8	39·8	40·5	None	Otoacoustic emissions	Normal	Normal	1·60 mmol/L	0·00	0·18	0·04	Midazolam	Midazolam
11	0·3	0·9	NA	11·6	Hearing loss, dehydration	Both automated auditory brainstem	20 dB	50 dB	1·47 mmol/L	0·00	0·72	0·10	None	None
12	0·2	0·8	20·1	5·6	None	Otoacoustic emissions	Normal	Normal	2·10 mmol/L	0·00	0·00	0·00	None	Phenytoin, PB
13	0·2	0·8	21·8	10·3	None	Otoacoustic emissions	Normal	Normal	NR	3·63	0·59‡	0·19‡	None	Phenytoin
14	0·2	0·8	23·3	23·0	Hearing loss	Otoacoustic emissions and automated auditory brainstem	90 dB	40 dB	NA	4·90	0·00‡	0·00‡	None	PB

Hearing loss grading: mild 20–40 dB, moderate 41–70 dB, severe 71–90 dB, profound >90 dB (normal thresholds are –10 to 20 dB). *Together with second dose of phenobarbital. †qIf screening was abnormal, second test was done between 17 and 108 days of age. ‡More than 80% reduction of seizure burden in min/h compared with baseline; baseline is not the same as inclusion criteria and therefore seizure burden baseline might be 0 whereas inclusion criteria had been met before. §Not applicable owing to death before hearing test. NR=not recorded. AED=antiepileptic drug. PB=further dose of phenobarbital. NA=not applicable.

Table 2: **Outcome measures of safety and efficacy**

dose of phenobarbital (n=4), and lidocaine (n=2). In four of five infants with an initial seizure reduction considered treatment success as per protocol, seizure burden increased within 5–10 h of the initial administration of bumetanide, and remained high enough to necessitate rescue drug. The primary outcome measure for safety was available for all included infants, although the period of safety assessment was reduced for five infants. Low blood pressure was reported in seven infants but in all cases was managed with adjustment of fluids. No major electrolyte disturbance was reported (table 3).

Of the 11 survivors, three failed the initial hearing screen (done between 8 and 27 days of life) and subsequent auditory brainstem responses confirmed hearing loss in all three cases (tables 2 and 3). Hearing loss was unilateral in one and bilateral (although asymmetric) in two infants, recorded at three different doses. One of the three infants had moderate and two had severe hypoxic ischaemic encephalopathy; two also received aminoglycosides, but neither had high trough levels or were treated for more than a week (one for 3 days, the other for 6 days). Bumetanide administration was done within 3–24 h (mean 10·5 h) of the aminoglycoside administration. The data monitoring committee reviewed data twice—after the inclusion of ten patients and after the third case of hearing loss. After

	At baseline	During trial
Major adverse reactions*		
Severe dehydration with hypotension	0	0
Severe hyponatraemia (but ≥120 mmol/L)	1	1
Severe unexpected serious adverse reaction	NA	0
Hearing loss	NA	3
Adverse events and reactions*		
Hypotension (mild to moderate)	6	7
Dehydration (mild to moderate)	0	1
Hyponatraemia (mild to moderate)	12	10
Hypokalaemia (mild to moderate)	6	7
Hyperglycaemia (mild to moderate)	3	3
Abnormal renal function (mild)	5	4
Haemoconcentration	0	0
Postnatal complications (adverse events†)		
Respiratory distress	9	9
Apnoea	0	1
Air leaks	2	0
Rash	0	1†
Fever	0	1
Prolonged coagulation times	9	4
Raised liver enzymes concentrations	8	5
Anaemia (mild to moderate)	4	0
Leucocytosis	0	4
Hyperbilirubinaemia	0	0
Hypoglycaemia (mild)	0	1
Major cause of death		
Related to hypoxic ischaemic encephalopathy	NA	3
Respiratory failure	NA	3‡
Coagulopathy	NA	1
Multiorgan failure	NA	1

*Events recorded during trial were considered at least possibly related to study drug. †Events recorded during trial were not considered related to study drug. †7 days after discharge from trial and therefore unlikely to be related to bumetanide. ‡Caused by redirection of intensive care in all three infants. NA=not applicable. More than one diagnosis is possible.

Table 3: **Adverse reactions and events**

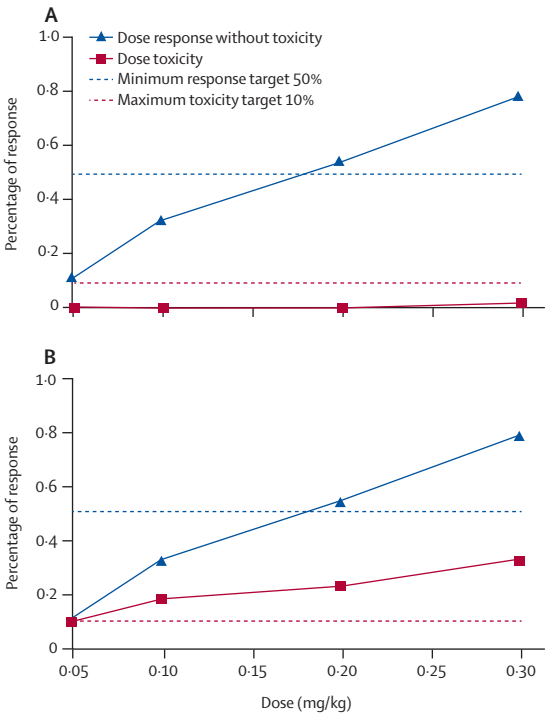

Figure 3: **Posterior estimated dose-response without toxicity and dose-toxicity associations at the end of the trial**

The posterior estimate is calculated with the endpoint as defined in the protocol and updated with the previous information with the data accumulated during the trial. (A) Posterior estimated dose–response curve (blue solid line) and dose–toxicity curve (red solid line) at the end of the treatment phase. Doses of 0·2 mg/kg and 0·3 mg/kg are estimated to be efficient. (B) Posterior estimated dose–response and dose–toxicity associations at the end of the trial after hearing tests. All doses are estimated to be toxic.

their recommendations and after review of the data, the trial steering committee decided to stop the trial early because of insufficient efficacy and a potential increased prevalence of hearing loss. Most other adverse events were related to hypoxic ischaemic encephalopathy (table 3). Three infants died; all three deaths were related to hypoxic ischaemic encephalopathy and all three babies had been withdrawn from the trial as a decision was made to withdraw intensive care within the first 3 postnatal days.

We noted no dose-limiting, adverse events during the trial. Figure 3 shows the posterior estimated association between dose and response without toxicity for 14 infants. According to the tolerance and efficacy data that were available at the end of the treatment period, two dose levels were estimated to be efficient and safe: 0·2 mg/kg was associated with an estimated posterior mean percentage of response without toxicity of 54·3% (95% credibility intervals 33·7–78·4%) and an estimated posterior mean percentage of toxicity of 0·4% (0–15·1%); and 0·3 mg/kg was associated with an estimated posterior mean percentage of response without toxicity of 78·7% (65·4–89·5%) and an estimated posterior mean percentage of toxicity of 1·3% (0–23·1%). The safe most successful dose was estimated to be 0·3 mg/kg, although the 0·2 mg/kg dose also fulfilled the minimum efficacy threshold. However, when we first estimated the safe most successful dose, the hearing loss tests were still not available. After this new information was taken into account, the posterior estimated association between dose and response at the end of the trial suggested that all doses are toxic (figure 3).

The final pharmacokinetic model was a two-compartment model with zero-order infusion and first-order elimination. The mean clearance, steady-state distribution volume, and elimination half-life of bumetanide were 0·063 L/h, 0·729 L, and 8·43 h, respectively (appendix p 4). Bodyweight explained the inter-individual variability of bumetanide elimination clearance via an allometric model (clearance

$[L/h]=0\cdot063\times(bodyweight/3\cdot4)^{1\cdot69}$. The association between bumetanide clearance and birth bodyweight retrospectively justified that bumetanide was given on a mg/kg basis.

Discussion

Bumetanide in doses up to 0·3 mg/kg, coadministered with a further dose of 10 mg/kg phenobarbital, resulted in five of 14 children meeting pre-specified EEG efficacy criteria and two of 14 children meeting rescue criteria, but none meeting both efficacy criteria (>80% seizure reduction and no need for rescue antiepileptic drugs). Apparent short-term tolerability was good, but subsequently the recorded proportion of hearing loss was higher than expected in infants with hypoxic ischaemic encephalopathy. Because of the adverse associations between dose and response and between dose and toxic effects, the trial was ended prematurely. These data suggest that the treatment of seizures in newborn infants with hypoxic ischaemic encephalopathy with bumetanide might increase the risk of hearing loss without evidence for improving seizure control. Careful safety assessments including interactions with other drugs, such as aminoglycosides, are necessary before considering further trials with bumetanide in this population.

Guidelines for the management of neonatal seizures lack an evidence base.[1] Unlike in adults, no new antiepileptic drugs have been developed for newborn babies, mainly because of ethical and logistical challenges, including the need for continuous EEG monitoring (panel).[2,3,19,20] The only published data for the anticonvulsive efficacy of bumetanide is one case report, in which one dose of bumetanide was associated with a reduced seizure burden of less than 50%.[21] In our study, bumetanide given with phenobarbital was associated with more than 80% reduction of seizure burden in less than half of infants and did not seem to affect the need for rescue drug. Additionally, reduction in seizure burden might have resulted from the coadministered second dose of phenobarbital[15] or the fluctuation in seizure burden noted in many infants with hypoxic ischaemic encephalopathy.[3] Coadministration was used to maximise the potential effect,[6] but is at the same time a limitation of the trial because the apparent short-term reduction in seizures might have been mediated by the additional phenobarbital. Lack of seizure reduction could be due to insufficient efficacy in human beings or restricted penetration of the blood–brain barrier because bumetanide is highly bound to protein and ionised at physiological pH.[7,8] Although the penetration of the blood–brain barrier is of great concern in older children and adults, this is less likely to be of relevance in newborn babies with global hypoxic injury because both (immaturity and hypoxic injury) are associated with reduced blood–brain barrier integrity.[6,22]

During and after a hypoxic insult, a fall in high-energy phosphate concentrations is recorded with rapid recovery, followed by a further fall around 6–18 h later associated with further cell death (secondary energy failure).[23] The severity of secondary energy failure has been related to subsequent adverse neurodevelopmental outcome.[23] Seizures start in the initial phase of secondary energy failure and typically express an evolving pattern, with rapid onset, peak incidence at 12–24 h of age, fluctuation in seizure burden, and a marked decrease or cessation at around 72–94 h.[3] Detection and quantification of seizure burden especially for assessment of antiepileptic drugs is possible only with continuous multichannel EEG monitoring.[2,3] Hypothermia has been shown to reduce the total seizure burden after hypoxic ischaemic encephalopathy but also increases fluctuation of the instantaneous seizure burden.[3] These fluctuations make the study of electrographic seizures as a primary outcome measure problematic if too short a period is used for comparison. Conversely, the use of rescue drugs and spontaneous reduction in seizure burden do not allow longer assessment periods. Our study emphasises that traditional outcome measures for the efficacy of antiepileptic drugs are not valid in the neonatal period. Therefore, innovative methods to assess the efficacy of antiepileptic drugs for neonatal seizures that take into account the typical fluctuating pattern observed are needed.

One of the limitations of this trial is the fact that five infants had no seizures during the baseline period (although they did have seizures during the inclusion period). According to good clinical practice, eligible

Panel: Research in context

Systematic review

We searched PubMed in Jan 2, 2015, for English language reports using the keywords "neonatal seizures", "antiepileptic drug", "EEG", and "clinical trial". We identified only two trials assessing safety and efficacy of antiepileptic drugs in the neonatal period with adequate methods including continuous EEG; both studied established antiepileptic drugs.[15,16] We found no published studies on the assessment of bumetanide in neonatal seizures or any other new antiepileptic drugs. The WHO guidelines[1] on neonatal seizures emphasise the paucity of evidence for the management of neonatal seizures. All recommendations made regarding choice of first-line and second-line antiepileptic drugs in the neonatal period were regarded as weak and based on low or very low quality of evidence. This results in frequent off-label usage of antiepileptic drugs in this population, including the use of bumetanide, despite a paucity of safety and efficacy data.[20]

Interpretation

To our knowledge this is the first study on the safety and efficacy of bumetanide for the treatment of neonatal seizures. We assessed pharmacokinetic data, dosage, and safety, but were unable to provide evidence for efficacy, partly because of problems relating to the interpretation of the evolution of seizures in this population. We stopped the trial because of the occurrence of hearing loss and lack of benefit in neonatal seizures. The results of our trial highlight the need for thorough safety assessments of new drugs in this vulnerable patient group, the challenges posed in undertaking trials of antiepileptic drugs in newborn infants, and the necessity for continuous EEG monitoring, innovative statistical and pharmacokinetic methods, and a multicentre and multidisciplinary approach.

patients should not be excluded from an intention-to-treat analysis; therefore, these patients were included in the analysis. Three of the five infants without seizures at baseline subsequently needed rescue drugs. We cannot exclude the possibility that bumetanide improves response to rescue drugs.

We recorded hearing loss in three (27%) of 11 surviving infants. Although of concern, these numbers are too small to show causation. No populations in the scientific literature are directly comparable to the NEMO cohort. Infants included in trials of therapeutic hypothermia all had moderate to severe hypoxic ischaemic encephalopathy but only a subgroup of these will have had refractory seizures. Nonetheless, the prevalence of hearing loss sufficient to require aids at 18 months of age was much lower (4–7%) than in our study.[24,25] Therefore, we did an audit of unselected patients with hypoxic ischaemic encephalopathy, which showed only one of 33 consecutive infants with moderate to severe hypoxic ischaemic encephalopathy had hearing loss (appendix p 5).

Hearing loss in babies with moderate to severe hypoxic ischaemic encephalopathy is difficult to study because of coincident risk factors, in particular administration of aminoglycosides, which happened simultaneously with bumetanide in two of three children with hearing loss. However, no infant had high trough aminoglycoside concentrations or associated early hypoglycaemia, which have been associated with hearing impairment,[26] and none of those with hearing loss carried the m1555AG mutation, which is associated with increased aminoglycoside sensitivity.[27] Thus, we believe caution is appropriate in any further attempt to show an anticonvulsant effect of bumetanide.

Similar to other loop diuretics, bumetanide is associated with a potential risk of ototoxicity but this risk is low in comparison with that from furosemide, especially if given via slow infusion;[8,28] ototoxicity has not been described in previous studies of neonatal safety.[8–10,12] Disruption of NKCC1 by bumetanide has been proposed as a cause of deafness by blocking the generation of the endocochlear potential, necessary for cochlear amplification.[29] By contrast, aminoglycosides can cause sensorineural hearing impairment by inducing hair cell loss after entering the cochlea and destroying hair cells via the caspase-dependent apoptotic pathway.[11] Loop diuretics can enhance uptake of cationic aminoglycosides into the endolymph by temporarily reducing the endocochlear potential.[30,31] This synergistic effect has been noted only in animals at doses very close to the median lethal dose (LD_{50}).[30] Temporal proximity in administration might be a contributing factor and coadministration should generally be avoided. Additional factors might increase susceptibility to hearing loss—eg, genetic idiosyncrasy, ischaemia, hypovolaemia, anoxia, noise, stress, and comorbidity (appendix p 9).[11,27,32] A multiple hit theory might explain the high proportion of infants with hearing loss in our trial. Even if aminoglycoside use could have been avoided, other factors are inescapable risk-enhancers for infants with seizures due to hypoxic ischaemic encephalopathy, but the drug might be safe without concomitant aminoglycoside use.

Drug trials in newborn babies pose major ethical dilemmas: balancing the potential risks and benefits of research against harm from inadequately studied treatments, leading to potentially effective treatments being witheld for lack of evidence. These results highlight the risk associated with off-label usage of drugs in newborn babies before safety assessment in controlled trials. Our findings also have implications for the design of future drug trials because they emphasise the logistical and ethical difficulties of doing studies in this patient group.

Contributors

All authors contributed to the design of the study, interpreted the data, critically reviewed and approved the manuscript. GP and VJ designed and analysed the pharmacokinetic section of the study. Clinical data acquisition was done by MB, LSdV, BH, BrM, RS, and MCT. Data analysis was by RMP, GBB, LSdV, NM, VJ, and SV. Statistical analysis was done by SZ and VL. RMP, GBB, and NM wrote the manuscript, with VJ adding the PK sections and SZ the statistic sections.

Declaration of interests

We declare no competing interests.

NEMO study group

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