N-ACETYLCYSTEINE DOES NOT PREVENT BRONCHOPULMONARY DYSPLASIA IN IMMATURE INFANTS: A RANDOMIZED CONTROLLED TRIAL

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Objective
To evaluate whether N-acetylcysteine (NAC) infusion during the first week of life reduces the risk of death or bronchopulmonary dysplasia (BPD) in infants with extremely low birth weight.

Study design
In a Nordic multicenter, double-blind trial, infants (n = 391) weighing 500 to 999 g and on ventilator or nasal continuous positive airway pressure were randomized before the age of 36 hours to receive NAC 16 to 32 mg/kg/d (n = 194) or placebo (n = 197) intravenously for 6 days. Primary end points were death or BPD, defined as supplementary oxygen requirement at 36 weeks' gestational age.

Results
There was no difference in the combined incidence of the primary end points death or BPD, 51% vs. 49%, between the NAC group and control group. Also similar was the incidence of BPD in survivors at 36 weeks' gestational age, 40% vs. 40%, and the mean oxygen requirement at the age of 28 days, 31.2% vs. 30.7%, respectively. The severity of BPD was similar in both groups.

Conclusions
A 6-day course of intravenous N-acetylcysteine at the dosage used does not prevent BPD or death in infants with extremely low birth weight. (J Pediatr 2003;143:713-9)

Antenatal glucocorticoids, surfactant replacement, and improved methods of intensive care and monitoring have markedly decreased the mortality of very preterm infants. However, bronchopulmonary dysplasia (BPD) remains an important cause of long-term morbidity after preterm birth. The pathogenesis of BPD is multifactorial, but among the key factors are oxygen therapy and inflammation. Both are known to induce increased production of reactive oxygen species and release of proinflammatory cytokines, which are capable of damaging cells and tissues as well as interfering with lung development. Appropriate ventilatory strategies to maintain optimal functional residual capacity, together with surfactant treatment, may reduce but not eliminate the risk of BPD. Glucocorticoids may acutely reduce ventilatory and oxygen requirements, but no long-term benefit in terms of BPD incidence and lung function in infants with extremely low birth weight has been shown, and an increased risk of brain damage as well as decreased growth has been reported. Evidence for inflammation, increased oxidant production, and oxidant-induced macromolecular damage has been demonstrated during the first week of life in infants developing BPD.

In contrast with many experimental animals, the enzymatic antioxidant defenses of preterm human neonates appear relatively well developed, with the exception of catalase. However, immature infants have a relative glutathione deficiency, which increases with decreasing gestational age. Given the dual role of glutathione as a cosubstrate in peroxidase reactions and as a direct scavenger of reactive oxygen species, such a deficiency...
may impair antioxidant defenses. The synthesis of glutathione does not appear to be limited by the activity of γ-glutamylcysteine synthetase even in preterm infants, but rather by the availability of cysteine. In this respect, preterm infants are at a disadvantage, because the transulfuration pathway from methionine into cysteine is not functional as a result of deficient activity of cystathionase. During parenteral nutrition of immature infants, cysteine is the first amino acid to show decreased plasma levels.

Several trials of antioxidant treatment of preterm neonates to prevent BPD have been performed, with varying success. Neither vitamin A nor vitamin E has a consistent effect in the risk of BPD or death in infants with extremely low birth weight. Intratracheal administration of copper-zinc-superoxide dismutase did not reduce the incidence of BPD, but there was less pulmonary morbidity at age 1 year.

Given the important role of glutathione in antioxidant defense and the relative lack of glutathione in preterm infants, a rational approach to treatment would be to replenish glutathione stores. However, glutathione itself crosses cell membranes poorly, and its rate-limiting precursor, cysteine, is unstable in solutions. N-acetylcysteine (NAC) is a precursor of cysteine and is by itself a free radical scavenger. Furthermore, it is available as a registered drug for intravenous use to treat acetaminophen poisoning. NAC enters the cell membrane poorly, and its rate-limiting precursor, cysteine, is unstable in solutions. N-acetylcysteine (NAC) is a precursor of cysteine and is by itself a free radical scavenger. Furthermore, it is available as a registered drug for intravenous use to treat acetaminophen poisoning. NAC enters the cell and is deacetylated to cysteine, which can replenish intracellular glutathione. NAC treatment reduced lung injury in adults with adult respiratory distress syndrome in some studies, but not in others.

We hypothesized that NAC would decrease morbidity and mortality in preterm infants because of its direct antioxidant effects and its ability to increase intracellular glutathione concentrations. The aim of this study was to evaluate whether NAC infusion during the first week of life reduces the risk of death or BPD in infants with extremely low birth weight.

**METHODS**

This randomized, double-blind, placebo-controlled, multicenter trial was performed in 10 academic neonatal intensive care units in Denmark, Finland, Iceland, Norway, and Sweden between March 1997 and April 2001. The research ethics committees of each institution and the National Agencies for Medicines in each country approved the protocol. Informed parental consent was obtained for each infant. Randomization was stratified for each center in blocks of 10 patients.

Infants with birth weights of 500 to 999 g who were on ventilator or nasal continuous positive airway pressure were eligible for enrollment before the age of 36 hours. Children with major congenital anomalies were excluded.

The infants were randomized either to intravenous NAC or to placebo group. For the first 67 patients, the preparations were Parvolex (N-acetyl-L-cysteine; 200 mg/mL, Evans, UK), or 0.9% sodium chloride. The remainder received Mucomyst (N-acetyl-L-cysteine; 200 mg/mL) or its solvent without NAC, manufactured in identical vials by Draco Läkemedel AB, Lund, Sweden. Sets of 10 vials for each patient were numbered by the Pharmacy of Helsinki University Central Hospital for each center according to the randomization list and were used in consecutive order. The NAC vials were diluted 1:100 to a final concentration of 2 mg/mL with 5% to 30% glucose solution, depending on the glucose requirement of the infant. The dilution procedure for the placebo infusion was identical. The infusion was started before the age of 36 hours and lasted for 6 days at a constant rate of 16 to 32 mg/kg/d, which was calculated on the basis of birth weight, with the smallest infants receiving the lowest dosages. This dosage was based on the results of our pharmacokinetic study of intravenous NAC in preterm babies and aimed at a steady-state plasma concentration of 100 to 300 μmol/L. The target concentration was based on a clinical study in adults with myocardial infarction and on our unpublished work on cultured cells exposed to oxidants.

The patients were treated according to the routine procedures in each intensive care unit and received parenteral and usually some enteral nutrition. Intravenous amino acids (Vaminolac, Fresenius-Kabi, Uppsala, Sweden, containing 1 mg/mL cysteine + cystine) were started on day 1 or 2, with the first dose 8 mL/kg/d, increasing daily to as much as 40 to 60 mL/kg/d. Ultrasound examinations of the brain were performed at least once during the first week and at the age of 28 days. To describe the radiological severity of BPD, chest radiographs were taken at the ages of 28 days and 36 gestational weeks and classified according to Weinstein et al and Toce et al by one radiologist. To ensure compliance and to evaluate drug metabolism plasma NAC, cysteine, and glutathione concentrations were assayed by using a high-performance liquid chromatography method with penicillamine as the internal standard. In short, disulfides were first reduced by dithiothreitol to free sulphydryls, which were then derivatized with monobromobimane. Hence, the values we report here are for total NAC and total cysteine, which include various NAC and cysteine-containing disulfides in addition to free monomeric NAC and cysteine. A reversed-phase C-18 column was used to separate the adducts, and quantitation was performed by using a fluorescence detector.

To detect possible side effects caused by NAC, laboratory tests, including hemoglobin, white blood cell count, thrombocytes, alanine aminotransferase, international standardized ratio of thromboplastin time, and serum urea level were followed during the first 2 weeks. Blood pressure, heart rate, and oxygen saturation were monitored continuously during the study period, with special attention to the mean arterial blood pressure during 1 hour before and the first 6 hours after the start of the infusion. Relevant complications were recorded daily according to the requirements of the drug company.

The primary outcome was death by 36 gestational weeks or BPD, defined as any supplementary oxygen requirement at 36 gestational weeks. The severity of BPD was classified according to a recent proposal. Secondary outcome measures were requirement of supplemental oxygen at the age of 28 days, duration of ventilator or nasal continuous positive airway pressure during 1 hour before and the first 6 hours after the start of the infusion. Relevant complications were recorded daily according to the requirements of the drug company.

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pressure treatment, weight gain, and the incidence of other reactive oxygen species-related diseases: intraventricular hemorrhage (IVH) classified according to the criteria of Papile et al., periventricular leukomalacia (PVL) according to the criteria of Trounce et al., necrotizing enterocolitis (NEC) grade III or higher (NEC needing surgical treatment) according to Bell et al., and retinopathy of prematurity (ROP) classified as the stage of disease in the more severely affected eye, based on the International Classification of Retinopathy of Prematurity.

Our sample size calculation was based on the statistics during a 2-year period of 1993 to 1995 in the Hospital for Children and Adolescents, Helsinki University Central Hospital, which showed that 46% of babies weighing <1000 g at birth either died or developed BPD by the time they reached the age of 36 gestational weeks. To detect a 33% reduction in BPD or death with NAC treatment with a power of 80% and a level of significance of 5% with a two-tailed test, the sample size was estimated to be 180 in each group.

All data for eligible enrolled infants were analyzed on an intention-to-treat basis. Baseline data for the treatment groups were compared by using the $t$ test for continuous variables or the Mann-Whitney $U$ test, as appropriate. Categorical baseline characteristics and outcomes were analyzed by using the $\chi^2$ test. All statistical tests were two-tailed. Logistic regression was used to analyze the outcomes. Data were analyzed by using the SPSS 10.0 statistical package (SPSS, Inc, Chicago, Ill).

### RESULTS

A total of 397 infants were enrolled in the study, but six infants were excluded from the analyses, one because of parental refusal and five because of major congenital anomalies diagnosed after the enrollment (one transposition of great arteries, two tetralogy of Fallot, one major ventricular septal defect, and one anal atresia). After the exclusion of these infants, 391 infants participated in the study: 194 in the NAC group and 197 in the placebo group. In two cases, the infusion was discontinued because of dosage error, and the code was opened (one NAC, one placebo). These cases are included in the analyses. There were no statistically significant differences in the baseline characteristics between the treatment groups (Table I).

There was no difference in the primary outcome of death or BPD at the age of 36 gestational weeks between the NAC and placebo groups (Table II). In the NAC group, 51% of the infants had BPD or had died, compared with 49% of infants in the placebo group (odds ratio, 1.0; 95% confidence interval, 0.7-1.6). Mortality at the age of 36 gestational weeks between the groups did not differ significantly: 18% in the NAC group, 14% in the placebo group. The incidence of BPD in infants alive at 36 gestational weeks was also similar. The severity of BPD was similar in the NAC and placebo group (Figure). When the infants were divided into two subgroups according to their birth weight (cutoff, 750 g; Table II) or gestational age (cutoff, 27 weeks; data not shown), no significant differences were noted in the incidence of BPD or death between the NAC or placebo treatments.

### Table I. Baseline characteristics of infants according to treatment assignment

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NAC (n = 194)</th>
<th>Placebo (n = 197)</th>
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<tbody>
<tr>
<td>Antenatal glucocorticoid therapy</td>
<td>171 (89)</td>
<td>175 (89)</td>
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<tr>
<td>Pre-eclampsia</td>
<td>48 (25)</td>
<td>57 (29)</td>
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<tr>
<td>Maternal infection</td>
<td>70 (36)</td>
<td>61 (31)</td>
</tr>
<tr>
<td>Outborn</td>
<td>23 (12)</td>
<td>15 (8)</td>
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<tr>
<td>Vaginal delivery</td>
<td>83 (43)</td>
<td>79 (40)</td>
</tr>
<tr>
<td>Male gender</td>
<td>95 (49)</td>
<td>99 (50)</td>
</tr>
<tr>
<td>Small for gestational age (&lt;2 SD)</td>
<td>55 (28)</td>
<td>67 (34)</td>
</tr>
<tr>
<td>Respiratory distress syndrome</td>
<td>166 (86)</td>
<td>166 (84)</td>
</tr>
<tr>
<td>Surfactant treatment</td>
<td>143 (74)</td>
<td>132 (67)</td>
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<table>
<thead>
<tr>
<th>Mean (SD)</th>
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<tbody>
<tr>
<td>Age of mother, y</td>
</tr>
<tr>
<td>Birth weight, g</td>
</tr>
<tr>
<td>Gestational age, wk</td>
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<tr>
<td>Apgar score 1 min</td>
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<td>Apgar score 5 min</td>
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<tr>
<td>Age at starting treatment, h</td>
</tr>
<tr>
<td>Fraction of inspired oxygen</td>
</tr>
<tr>
<td>Mean airway pressure, cm H2O</td>
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<td>Oxygenation index</td>
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*Data are presented as percentages and means (SDs). There were no significant differences between the groups.
At the age of 28 days, there was no significant difference in mortality or need of supplemental oxygen. Also, the mean percentage of oxygen required at the age of 28 days was similar: 31.2% in the NAC group and 30.7% in the placebo group. There was no statistically significant difference in the need of respiratory support between the groups. The median duration of ventilator treatment was 5 and 6 days, the median duration of nasal continuous positive airway pressure treatment was 24 and 27 days, and the median duration of any mechanical respiratory support was 37 and 42 days in the NAC and placebo groups, respectively.

The incidences of the other outcome measures are presented in Table III. There were no significant differences in the incidence of IVH, NEC, patent ductus arteriosus, PVL, or ROP between the two groups, ascertained at death or at the age of 36 gestational weeks. Also, the weight gain from birth to the age of 36 gestational weeks was similar in both groups. The use of postnatal steroids did not differ between the NAC and placebo groups.

Chest radiographs were taken of 260 infants at the age of 28 days and in 230 infants at the age corresponding to 36 gestational weeks. No difference was found at either age: the scores according to Weinstein et al21 at 36 weeks were 3.0 ± 1.3 and 2.9 ± 1.2 and the scores according to Toce et al22 were 4.4 ± 2.0 and 4.1 ± 1.8 in the NAC and placebo groups, respectively.

In the patient group receiving NAC, the mean plasma concentration of NAC was 170 μmol/L (range, 0-548 μmol/L) on day 3 and 172 μmol/L (range, 0-351 μmol/L) on day 7, just before the 6-day infusion was stopped. On day 3, the mean plasma cysteine concentration was 108 μmol/L (range, 46-243 μmol/L) in the NAC group and 125 μmol/L (range, 44-253 μmol/L) in the placebo group (P = .01). On day 7, the mean plasma cysteine level was 144 μmol/L (range, 37-358 μmol/L) and 169 μmol/L (range, 40-390 μmol/L) in the NAC and placebo groups, respectively (P = .02). Although there was a difference between the group means, the increase in the plasma cysteine concentration from day 3 to day 7 was similar. The mean plasma glutathione concentrations were 19 μmol/L (range, 2-68 μmol/L) and 21 μmol/L (range, 2-59 μmol/L) on day 3, and 19 μmol/L (range, 2-33 μmol/L) and 23 μmol/L (range, 2-51 μmol/L) on day 7 in the NAC and placebo groups, respectively.

No differences were found between the treatment groups in blood pressure or in laboratory tests during the first 2 weeks of life. No adverse effects were observed that could be ascribed to NAC.

**DISCUSSION**

Our trial showed that a 6-day course of intravenous NAC did not prevent death or bronchopulmonary dysplasia in infants with extremely low birth weight. The reasons for this outcome can be sought either in a faulty basic rationale for the study or in the design and implementation of the trial. An
optimal strategy to prevent lung injury should be based on an understanding of its pathogenesis.

In the infant with extremely low birth weight, structural immaturity and surfactant deficiency necessitate mechanical ventilation and oxygen administration, both of which contribute to an inflammatory response. Oxidative stress in preterm infants is indicated by lower reduced and higher oxidized glutathione concentrations in plasma of preterm infants compared with term infants, and lower reduced glutathione levels in tracheal aspirates of preterm infants developing BPD.

Glutathione peroxidase is important for removal of intracellular hydrogen peroxide and lipid peroxides, and glutathione is a direct scavenger of oxidants both intracellularly and extracellularly. Its role in lung protection is suggested by the high concentrations of glutathione in the lining fluid of lower airways and alveoli, with a transient postnatal decrease in preterm infants. NAC may not be the most effective glutathione precursor, but it is available as a registered drug. Furthermore, NAC itself is a potent scavenger of the hydroxyl radical and hypochlorous acid, is somewhat slower to react with hydrogen peroxide, and is unreactive with superoxide.

In animal experiments, intraperitoneally administered NAC ameliorated hyperoxic lung injury in guinea pigs. Lipopolysaccharide-induced lung damage in rats was also attenuated by NAC, apparently because of scavenging of free radicals and inhibition of neutrophil oxidant production. Human studies of both the biochemical and clinical effects of NAC have been inconclusive or conflicting, but the trials have been small. In adult respiratory distress syndrome, NAC improved oxygenation and lung function but had no significant effect on mortality. Apart from acetaminophen poisoning and genetic disorders of glutathione metabolism, intravenous NAC has not been studied in children. A clinical trial to test the applicability of NAC for prevention of BPD thus seemed warranted.

The subjects of our trial, infants weighing <1000 g at birth, represent a high-risk group with respect to the primary outcome measures, and a reasonable one third reduction in the incidence of death or BPD was the basis for sample size calculation. The target number of patients was recruited, and thus, a significant effect of NAC on these outcomes was ruled out. However, because the incidence of other complications of immaturity is lower, potential beneficial effects of NAC on IVH, NEC, PVL, and ROP cannot be evaluated on the basis of this trial, even though the incidence of PVL was 39% higher in the control group than in the NAC group. A considerably larger trial would be required to demonstrate a beneficial effect of NAC on hypoxic-ischemic brain injury in newborn infants, which has been shown in experimental animals.

N-acetylcysteine infusion was timed over the 6-day period, during which several biochemical indicators of oxidant effects increase in plasma, in tracheal aspirates, and expired air. The largest change in the oxygen environment of the infant occurs immediately after birth, and usually at that time, ventilatory assistance is also initiated. An earlier start of NAC treatment could therefore have been more effective, but on the other hand, the trial would have recruited several infants who were weaned from the ventilator by age 36 hours. Whether a longer intervention could have been effective remains unclear.

To scavenge free radicals effectively and provide substrate for glutathione synthesis, an adequate dose of NAC must be given. There are no guidelines for the use of NAC in immature infants, and the desired therapeutic effect must be weighed against the risk of side effects. The dosage regimen based on our pharmacokinetic study succeeded in maintaining the target concentration of 200 μM in most of the infants. Whether a higher dose would have influenced the primary outcome measures is a matter of speculation. Side effects noted in patients treated for acetaminophen poisoning include hypotension, bronchospasm, and a decrease in blood pressure.
coagulation factors, but no adverse effects attributable to NAC were observed in our trial.

The scavenging function of NAC is exerted by the intact molecule, but it must be deacetylated to cysteine to act as a glutathione precursor. Adult human liver and endothelial cells are able to deacetylate NAC,37 but there is no information on the development of N-acetylase activity in any human tissue. Because in our study the change in plasma total cysteine concentration during the NAC infusion did not differ from that in the placebo infants, intravenous NAC may be poorly deacetylated in preterm infants. In a study of intravenous amino acid solutions for preterm infants, more than half of the NAC dose was excreted unchanged, and it had no demonstrable effect on plasma cysteine levels.38 Our unpublished studies have shown that NAC is able to reduce cysteine from cystine and from mixed disulfides. Thus, even if NAC itself were a poor substrate for glutathione synthesis, its potential benefits as an antioxidant would depend on its ability to scavenge oxidants chemically and to liberate cysteine from disulfides. Glutathione is mainly intracellular, and the goal for NAC treatment was to increase glutathione concentration in the cells. This could unfortunately not be studied. NAC infusion did not increase plasma glutathione or cysteine concentrations. The question remains open whether interventions increasing intracellular glutathione levels protect from the development of BPD.

In conclusion, no beneficial effects of NAC with the dosage used could be demonstrated at 36 weeks. An ongoing follow-up of the surviving infants will show whether the long-term pulmonary outcome can be improved with early NAC treatment, as was recently reported for infants treated at birth with recombinant human CuZn superoxide dismutase.12

REFERENCES


50 Years Ago in The Journal of Pediatrics

A CURRENT SUMMARY OF STRABISMUS IN CHILDREN
Regnier E. J Pediatr 1953;43:732-9

Strabismus, defined as misalignment of the eyes, is a common ocular disorder in childhood that can result in impaired vision. Although in the title of the article, Dr Regnier uses the contemporary word strabismus, the author uses throughout the article the earlier term squint in reference to evaluating pediatric patients "whose eyes are not straight." Referring to the Oxford English Dictionary, the word "squint" dates back to 1652. In the 1950s, it was generally used among the medical profession. Perhaps, because of its negative connotations in the lay community, it has currently lost favor. The author also uses the term "retrolental fibroplasias" in his review of strabismus in children. The current terminology is retinopathy of prematurity. Retrolental fibroplasia describes advanced cases of the disease entity in which the retina has become detached and has contracted behind the lens of the eye. Today, this advanced stage of the disease rarely occurs because of progress in prevention and treatment.

The author includes a cogent discussion of esotropia, exotropia, amblyopia, abnormal retinal correspondence, and fusion. When discussing surgery, the author points out that two days of hospitalization is usually sufficient. Today, the vast majority of strabismus surgeries are performed on an outpatient basis. The author also states that operations on the ocular muscles is feasible at one year, because earlier the risk of anesthesia is too dangerous. With advances in anesthesia, strabismus surgery can be safely performed as early as three months of age.

The author lists numerous types of strabismus that cannot be successfully treated in 1953, including strabismus related to a marked difference in refractive error, commonly referred to as anisometropia. For example, if a child has essentially no refractive error in one eye and is markedly myopic in the other, the myopic eye is likely to become amblyopic. Currently, contact lenses are often used to treat this condition. Another potential treatment for this condition would be laser-assisted in situ keratomileusis (LASIK), a surgical procedure in which a flap is created in the cornea with a microkeratome and a laser is used to reshape the underlying corneal bed. Currently, most LASIK procedures are performed in adults to correct nearsightedness. Theoretically, LASIK should be highly effective in treating children with markedly different refractive errors. Although LASIK is not currently approved by the FDA, preliminary LASIK studies in children for this condition appear encouraging. In contrast to adults in which LASIK is performed with topical anesthetic drops, LASIK in children is usually performed with general anesthesia.

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N-Acetylcysteine Does Not Prevent Bronchopulmonary Dysplasia in Immature Infants: A Randomized Controlled Trial