Intravenous immunoglobulin prophylaxis in neonates on artificial ventilation

M. Adhikari, A. G. Wesley, P. B. Fourie

The efficacy of the prophylactic use of intravenous immunoglobulin (Ig) was evaluated in a double-blind placebo-controlled trial of 21 pairs of ventilated neonates weighing more than 1 500 g. Each infant received 0.4 g/kg/day of intravenous Ig or a similar volume of placebo daily for 5 days. Criteria used to assess the efficacy of intravenous Ig were the number of infections, the duration of ventilation therapy and time to clinical recovery. There were no significant differences in the treated and placebo groups with regard to the frequency of positive blood cultures (28.6% and 14.3%), endotracheal cultures (57.1% and 66.7%) and abnormal white cell counts (52.4% and 57.1%). On entry to the study there was no significant difference in IgG levels between the treated (974.5 mg/dl; SD 575.3) and placebo groups (818 mg/dl; SD 516.9). However, on day 6 the treated group had a mean level of 1 400.3 mg/dl (SD 426.7) versus 710.9 mg/dl (SD 377.4) in the placebo group ($P < 0.05$). Clinical improvement occurred within 3 days in both groups. Ventilatory support was required for 11.8 days (SD 8.3) in the treated and 11.8 days (SD 7.3) in the placebo group. Both groups required 3 - 4 antibiotic treatments over a period of 14 - 15 days. Two patients died in the treated and 4 in the placebo group, with 1 infant in each group developing bronchopulmonary dysplasia. The patients who recovered did so within 14 days. Analyses of subgroups of patients with different diagnoses revealed no differences except a trend suggesting fewer infections in term babies treated with intravenous Ig. The organisms cultured in the intravenous Ig groups were *Pseudomonas, Klebsiella*, *Escherichia coli* and *Staphylococcus* and in the placebo group *Pseudomonas, Klebsiella* and *Enterobacter.*

The above has shown that, except for a trend in the older neonates, intravenous Ig is not of prophylactic benefit in ventilated neonates. Newer adjuncts in immunotherapy such as hyperimmune gammaglobulin or monoclonal antibodies may prove of greater value in the treatment of neonatal sepsis.

S Afr Med J 1996; **86:** 542-545.

Department of Paediatrics and Child Health, University of Natal and King Edward VIII Hospital, Durban

M. Adhikari, M.B. CH.B., F.C.P., M.D.

A. G. Wesley, M.D., F.R.C.P., D.C.H.

National Tuberculosis Research Programme of the Medical Research Council, Pretoria

P. B. Fourie, M.SC., PH.D.

Nosocomial infections remain a major hazard and contribute to morbidity and mortality in neonates managed in intensive care wards.[1] The incidence of secondary infection in high-risk neonates in the neonatal unit at King Edward VIII Hospital is 20 - 25%, with a mortality rate of 25%. A resistant *Klebsiella pneumoniae* has been responsible for a number of the nosocomially acquired infections in the unit for some time. However, the incidence of infections due to this organism has been declining. The factors contributing to the increased tendency of the neonate to acquire nosocomial infections are numerous.[2] Hypogammaglobulinaemia, more marked in preterm than full-term infants, may be an important contributor to this susceptibility.[1] The action of intravenous immunoglobulin (Ig) simply raises the IgG levels to boost antibody action, or to induce an opsonophagocytic effect, as shown *in vitro* in neonatal animals.[3] When this study commenced in 1987, the majority of patients admitted to the neonatal intensive care unit were babies with a birth weight greater than 1 500 g. Tetanus and meconium aspiration are common conditions in term babies admitted to the respiratory and neonatal intensive care units respectively. Facilities are very limited and are offered to those babies of higher gestational age and weight. We report a double-blind placebo-controlled trial of the prophylactic use of intravenous Ig (Sandoglobulin; Sandoz) in preventing nosocomial bacterial infection in ventilated neonates weighing more than 1 500 g at birth.

Methods

Neonates were selected for the study from the neonatal and respiratory units at King Edward VIII Hospital, Durban. During the period August 1987 - December 1988 infants with birth weights more than 1 500 g who required ventilation and had been diagnosed with hyaline membrane disease, meconium aspiration, pneumonia or severe tetanus within the first week of life were studied. Each randomly selected patient was matched to a control by weight, and by gestational and postnatal age. The weights were categorised in 500 g intervals from 1 500 to 4 000 g and the gestational ages from 28 to 40 weeks. Infants with severe malformations, severe birth asphyxia or overt sepsis were excluded.

Informed consent having been obtained, infants were allocated to receive either the intravenous Ig in a dose of 0.4 g/kg/day or an equivalent volume of placebo infused over 4 - 6 hours for 5 days. The intravenous Ig or placebo was supplied in identical bottles and the code was known to only one of us (P.B.F.), who had no contact with the patients. The intravenous Ig was supplied by Sandoz Products.

The clinical information noted in each infant included routine monitoring of heart and respiratory rates, blood pressure, arterial blood gases and blood sugar. Temperature instability was diagnosed when there was a change of 1°C above or below 37°C. The need for respiratory support was measured as the number of days for which oxygen and ventilation were required.

Peripheral venous lines only are used in our unit and repeat arterial stabs are undertaken for arterial blood gas analysis. Feeding took place via a nasogastric tube, either by continuous or intermittent gavage. Mother's own fresh milk was given almost exclusively. Where this was not possible, a milk formula was given. The diagnosis of sepsis was made on the basis of clinical manifestations, culture and haematological results.

The progress or appearance of pneumonia was graded on radiographs as 'improvement', 'no change' or 'deterioration'. Immunoglobulin levels were assessed before treatment, the day after the last infusion and on day 14. The full blood count, blood and endotracheal cultures were performed and chest radiographs were taken on admission to the intensive care unit, repeated every 3rd day and at any time in between when sepsis was suspected. Once the initial clinical and laboratory evaluations has been completed, all the babies received penicillin and an aminoglycoside.

Antibiotic therapy was then changed according to the organism cultured and its sensitivity. Further, if the chest radiographs indicated deterioration, instability of the temperature occurred or the white cell counts altered, antibiotic therapy was reviewed. A positive culture of endotracheal secretion alone (i.e. negative blood culture and normal white cell count) was considered indicative of colonisation and not treated with antibiotics. All interventions, such as the need to administer plasma or blood, or change antibiotics, were carefully noted. The half-life of intravenous Ig is 14 days and for the purposes of the study the patients were observed for a total of 21 days. Bronchopulmonary dysplasia was defined as oxygen dependency for longer than 28 days following an initial period of ventilation and compatible chest radiographs.[4]

Criteria for efficacy of intravenous Ig were the number of infections as measured by temperature instability, abnormal white cell counts (D1 < 5 000 or > 25 000; D4 < 5 000 > 12 000 x 10^9/l), positive cultures of blood and endotracheal aspirates, duration of time to clinical recovery, degree of ventilatory support required, number of antibiotics and duration of therapy. Ig levels were measured by laser nephelometry.

Statistical methods

Statistical analysis was performed with Statistical Analysis System (SAS) version 5 and included the paired-difference *t*-test for continuous and the Wilcoxon signed rank test for categorical data. Continuous variables included gestational age, age upon entry into the study, weight, IgG level, period of ventilatory support and time to recovery. All these variables are normally distributed in the population. Clinical diagnosis, sex and overall outcome were analysed with the chi-square test. All other variables, being ordinal or interval, were analysed with the Wilcoxon signed rank test. In all instances differences were regarded as significant at the 0.05 level.

Results

The characteristics of 21 pairs of neonates studied are shown in Table I. The clinical diagnosis, gender, gestational age, weight and age of entry to the study are also shown. Babies with neonatal tetanus presented at 6 - 7 days of age, influencing the age of enrolment. The neonates without tetanus were enrolled within 12 - 24 hours. There are no significant differences in the treated and placebo groups. Table II shows the culture results, white cell counts and immunoglobulin levels of the 21 pairs studied. The mean IgG

levels in the 11 pairs of babies with tetanus - pneumonia were 1 176 mg/dl (range 112 - 2 424) v. 1 193 mg/dl (range 964 - 1 224) at the outset, 1 365 mg/dl (range 235 - 1 786) v. 866 mg/dl (range 665 - 1 224) on day 6, and 862 mg/dl (range 633 - 1 224) on day 14 in the treated and placebo groups respectively.

Table I. Patients' details on entry to trial

	Gammaglobulin (N = 21)	Placebo (N = 21)
Clinical diagnosis†		
HMD	10	10
Tetanus	7	7
Pneumonia	4	4
Sex†		
Male	13	10
Female	8	11
Gestational age* (wks)		
Mean	37.1	37.0
SD	3.7	3.7
Age on entry* (d)		
Mean	5.7	5.6
SD	7.1	7.4
Weight* (g)		
Mean	2 702.4	2 679.1
SD	781.7	758.7

HMD = hyaline membrane disease.
Distribution for gammaglobulin and placebo groups similar for all variables.
† Chi-square test.
* Paired t-test: $P > 0.05$.

Table II. Laboratory findings

			Gammaglobulin (N = 21)	Placebo (N = 21)
Blood cultures†				
Negative			15	18
1 positive			4	2
2 or more positive			2	1
ETT cultures†				
Negative			9	7
1 positive			8	9
2 or more positive			4	5
WCC†				
Normal			10	9
1 - 2 abnormal			10	8
3 or more abnormal			1	4
IgG* mg/dl	Day 0	Mean	974.5	818.5
		SD	575.3	516.9
	Day 6	Mean	1 400.3	710.9
		SD	426.7	377.4

Distributions for gammaglobulin and placebo groups similar for all variables except for IgG.
† Wilcoxon signed rank test.
* Paired t-test: $P > 0.05$.
IgG differences between means:

Between groups	Day 0	T = 0.78	P = 0.45
	Day 6	T = 4.65	P = 0.0004
Within groups (days 0 and 6)	Gammaglobulin	T = 2.59	P = 0.02
	Placebo	T = 0.92	P = 0.37

In the 10 pairs of low-birth-weight infants with hyaline membrane disease, the mean IgG levels were 870 mg/dl (range 531 - 1 220) v. 913 mg/dl (range 745 - 1 110) on day 0, 1 436 mg/dl (range 1 020 - 1 673) v. 880 mg/dl (range 551 - 1 108) on day 6, and 1 363 mg/dl (range 1 020 - 1 640) v. 943 mg/dl (range 640 - 2 540) in the treated and placebo groups respectively. Assessment of the clinical parameters, management and recovery is shown in Table III.

Two patients in the treatment group and 4 in the placebo group died, with 1 infant in each group regarded as unresponsive, i.e. they had developed bronchopulmonary dysplasia. Patients who recovered did so within 14 days, i.e. 328 hours (SD 211) in the treated and 340 hours (SD 161) in the placebo group.

Table III. Clinical assessment and management outcome

	Gammaglobulin (N = 21)	Placebo (N = 21)
Ventilatory support* (d)		
Mean	11.8	11.8
SD	8.3	7.3
Clinical recovery† (h)		
Mean	328.2	339.8
SD	211.1	161.0
Outcome†		
Recovered	18	16
Unchanged	1	1
Died	2	4

Distribution for gammaglobulin and placebo groups similar for all variables.
† Chi-square test.
* Paired t-test: $P > 0.05$.

An analysis of a more uniform subgroup of 10 pairs who had hyaline membrane disease and meconium aspiration confirmed the findings in the 21 pairs. Similarly, analysis of a subset of 11 pairs with tetanus neonatorum or with pneumonia (7 pairs and 4 pairs respectively), showed no effect of intravenous Ig in the treated compared with the placebo group. However, it is interesting to note that all deaths occurred in the latter group.

The intravenous Ig was well tolerated by all the patients and no adverse effects were noted. The organisms cultured in the treated group were *Pseudomonas* species, *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus*. In the placebo group, *Pseudomonas* species, *K. pneumoniae* and *Enterobacter* were cultured.

Discussion

Despite the advances in neonatal intensive care, and as a consequence thereof, a significant percentage of low-birth-weight neonates experience nosocomial infections. Exchange transfusion,[5] granulocyte transfusions,[6] intramuscular human antibodies to endotoxin[7,8] and, most appealingly, intravenous Ig[1,9-11] have been used as adjuncts to antibiotics and as supportive measures. Intravenous Ig has been given prophylactically to low-birth-weight infants for the prevention of early[1,9,11] and late sepsis.[10]

In this study prophylactic intravenous Ig for early sepsis did not significantly reduce the rate of infection, the duration of ventilation or the time to clinical recovery. A further analysis of a subgroup of 10 pairs of low-birth-weight infants with hyaline membrane disease and a second group of term infants with tetanus and pneumonia has shown no advantage to the low-birth-weight group receiving intravenous Ig. However, there was a trend towards a decreased number of infections and fewer deaths in the neonates with higher body weights. From the current

literature,[11] term babies seem to be less likely to benefit, but at the commencement of this study, the evidence of a possible advantage of intravenous Ig in low-birth-weight infants was less clear. During the course of this study, a decline in nosocomial infection was noted and, having observed no significant advantage of intravenous Ig in the patients studied, we elected to discontinue the study. Unfortunately, the sample size necessary for a 50% reduction in the sepsis rate of 25% with a power of 80 - 90% exceeds the 21 patients and carefully matched controls in this sample. The required number could not be found in a reasonable period of time and would require a multicentre trial. Further, the analysis of these data suggested that no significant advantage of intravenous Ig would be shown with a larger sample. Baker *et al.*[12] calculated that for an infection rate of 15 - 20%, 500 - 700 low-birth-weight infants would have to be evaluated to assess the efficacy of prophylactic intravenous Ig.

An important aspect of this study is the level of IgG in the groups studied. In both groups the pre-treatment evaluation of the IgG levels revealed levels above the 'protective' target level suggested by Kyllonen *et al.*[13] This implies that the value of IgG levels may not be as important as initially thought, but that the opsonophagocytic effect[3] may need further investigation. The total dosage of intravenous Ig administered in this study is similar to those in other studies.[1,10] A higher dose of intravenous Ig may result in an antagonistic effect due to blockage of the reticulo-endothelial system and may have an adverse effect on the outcome.[13] A study conducted in Cape Town revealed that a single high dose of intravenous Ig (1 g/kg) did not alter the morbidity, mortality or duration of neonatal intensive care of neonates weighing less than 1 500 g and under 34 weeks' gestational age.[14]

The results of a number of studies have provided inconclusive evidence of the advantage of intravenous Ig. One study of infants weighing less than 2 000 g, the prophylactic administration of intravenous Ig for early sepsis showed a possible advantage.[11] Other studies demonstrated a clear reduction in infection in those weighing less than 1 500 g, and no effect on the incidence of infection in those of a higher birth weight.[1,9] Prophylactic intravenous Ig for late-onset sepsis revealed no clear advantage in babies weighing less than 1 300 g.[10] The only study on the use of intravenous Ig conducted in the developing world with an incidence of bacterial infection comparable to our study's showed that prophylactic intravenous Ig for early infection was of benefit compared with controls.[9] Gonzalez and Hill[3] evaluated all the studies in which intravenous gammaglobulin was used in human neonates and concluded that none of the studies had satisfactorily demonstrated the efficacy of intravenous Ig as a therapeutic or prophylactic agent. However, most studies, including animal studies, suggest a beneficial effect.

In a study of human immunoglobulins, a comparison was made of available preparations of immunoglobulins for group B streptococcal antibody levels and opsonic activity. In mice given a lethal challenge of group B streptococci, the pH-treated intact 7S intravenous Ig preparation (Sandoglobulin) was the most effective in reducing mortality.[15] However, also to be considered is the variation in protective activity, i.e. opsonic activity that enhances phagocytosis among batches of Sandoglobulin.[15] That Sandoglobulin is similarly effective against the organisms found in this study (*K. pneumoniae, E. coli, S. aureus*) has not been shown. The studies of intravenous Ig have led to the further development of immunotherapy in the newborn. At present it would appear that specific immunotherapy for individual organisms is a more appropriate therapeutic strategy than a less specific, very expensive form of therapy such as intravenous Ig. For example, hyperimmune gammaglobulin for group B streptococci,[16] or group B streptococci vaccines[17] may prove to be of value in this infection. Further progress in immunotherapy has been the development of a monoclonal antibody specific for the K, capsule of *E. coli*[18] and another one that reacts with all five types of group B streptococci.[19] The results of these two studies suggest that administration of small amounts of human monoclonal antibodies may be highly specific and directly protective against these organisms. With time, monoclonal antibodies to all organisms may be developed and may prove to be of greater value in controlling neonatal sepsis.

We wish to thank the Medical Superintendent of King Edward VIII Hospital for permission to conduct this study. We thank Jeanette Allan for her assistance, and Sandoz for providing the Sandoglobulin and the financial support for the research assistants. We are also very grateful to Mrs E. Yolland, Mrs K. van Rooyen and Miss R. Seetal for typing the manuscript. We are also indebted to our colleagues, in particular Drs P. K. Desai and M. Pather, who assisted us with the clinical investigations.

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Accepted 16 Aug 1994.