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Confronto tra due protocolli con Eritropoietina Umana Ricombinante (rHuEpo) nel trattamento dell'anemia tardiva nei neonati con Isoimmunizzazione Rh.

Comparison between two treatment protocols with recombinant Human Erythropoietin (rHuEpo) in the treatment of late anemia in neonates with Rh-Isoimmunization

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Riassunto

Obiettivi. La malattia emolitica del neonato da isoimmunizzazione Rh può portare ad una anemia sia su base emolitica che iporigenerativa. Abbiamo confrontato l'efficacia dell'Eritropoietina Umana ricombinante (rHuEPO) in due protocolli che si differenziano per le dosi e per la tempistica di somministrazione dell'rHuEpo.
Metodi. È stata studiata una coorte di 14 neonati. I neonati sono stati trattati con due diversi protocolli. Protocollo A: una dose di 200 U/kg/die di rHuEPO somministrata per via sottocutanea a partire dalla fine della seconda settimana di vita. Protocollo B: una dose di 400 U/kg/die di rHuEPO somministrata per via sottocutanea a partire dalla fine della prima settimana di vita.
Risultati. I valori di ematocrito nel gruppo del protocollo A sono diminuiti durante il trattamento (32,5% vs 25,2%), mentre il valore di ematocrito nel gruppo del protocollo B è rimasto pressoché stabile (38,7% vs 42,8%). Il numero medio di piastrine è rimasto stabile in entrambi i gruppi, mentre si è evidenziato un aumento dei neutrofili nel gruppo del protocollo A e una diminuzione nel Protocollo B (p<0,05). La conta dei reticolociti è aumentata durante il trattamento in entrambi i gruppi, anche se solo nel gruppo del protocollo B è stata statisticamente significativa (p <0,05).
Conclusioni. I nostri risultati suggeriscono una efficacia simile tra i due protocolli di trattamento. Un aumento delle dosi di rHuEPO non sembra migliorare l'efficacia e l'incidenza di effetti collaterali.

Abstract

Objectve. The Rh-hemolytic disease can lead to a late anemia by hemolytic and hyporigenerative mechanism. We compared the effectiveness of rHuEPO in two care protocols that differ for doses of rHuEPO administrated and for timing of administration.
Methods. A cohort of 14 neonates was investigated. The neonates were treated with two different protocols. Protocol A: a dose of 200 U/kg/day of rHuEpo administered subcutaneously starting from the end of the second week of life; Protocol B: a dose of 400 U/kg/day of rHuEpo administered subcutaneously starting from the end of the first week of life.
Results. The hematocrit values in the protocol A group decreased during treatment (32,5% vs 25,2%), whereas the hematocrit value in protocol B group remained almost stable (38,7% vs 42,8%). The mean numbers of platelets remained stable in both groups while neutrophils increased in protocol A group and decreased in protocol B (p<0,05). Reticulocyte count increased during treatment in both groups, although only in protocol B group it was statistically significative (p<0,05).
Conclusions. Our results suggest a similar efficacy between the two treatment protocols. Increasing doses of rHuEPO do not seem enhancing their effectiveness and the incidence of side effects.

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Introduction

Since 1989 Koenig JM et al.[1] distinguished two kinds of late anaemia in neonates with Rh-isoimmunization.
The first type of anaemia develops between the second and the third week of life. It is a progressive anaemia, that presents high levels of reticulocytes and accelerated medullary production of normoblasts. The second type of anaemia develops between the fourth and the sixth week of life and it is characterized by a low level of haemoglobin, reticulocytes and plasmatic erythropoietin. It is more frequently detected in neonates that received one or more intrauterine transfusion (IUT).
Many pathogenetic mechanisms have been identified by literature.
The principal mechanisms are: persistent hemolysis due to anti-D antibodies,[2,3] erythroid hyporegeneration caused by the presence of anti-D antibodies in the bone marrow which destroy erythroid precursors[4,5] and erythroid hypoplasia already present at birth[6] due to high concentrations of hemoglobin-A introduced during IUTs.[4,5,7-11]
This study focuses on the effects of rHuEpo (recombinant human Erythropoietin) in 14 intrauterine-transfused neonates treated with two care protocols that differ for doses of rHuEPO administrated and time of administration. Moreover we searched for any possible side effect of the rHuEPO treatment.

Materials and methods

A cohort of 14 neonates born between June 1994 and November 2006 admitted to the wards of Neonatal Patology and Neonatal Intensive Care Unit of the Agostino Gemelli Polyclinic was investigated. All neonates were affected by fetal or neonatal Rh-isoimmunization.
Gestational age was determined by considering the date of the last menstrual cycle or by a first trimester ultrasound. Birth weight percentile was calculated using the Italian growth curve[12]; a small for gestational age (SGA) designation was defined as a neonatal weight less than the 10th percentile. The need for an IUT and the volume of packed filtered red cells (PFRCs) to be transfused were decided according to the standard protocols by using a nomogram and by taking as reference the studies of Nicolaides et al. and Plecas et al.[13,14] All IUTs were performed through cordocentesis by using Rh-negative PFRCs.
All neonates were treated with rHuEpo for the prevention of late anemia. They received IUT or exchange transfusions (EXTs) and/or blood transfusion (ET) after birth because they developed anemia and reticulocytopenia. All were inborn; a few ones were monitored as outpatient after discharge, by performing blood counts and blood chemistry to monitor the therapy in progress. The rHuEpo was administered by the nursing staff of the ward in hospital.
The neonates were treated with two different protocols: six were treated according to protocol A that was characterized by a dose of 200 U/kg/day of rHuEpo administered subcutaneously starting from the end of the second week of life, eight neonates were treated according to protocol B that was characterized by a dose of 400 U/kg/day of rHuEpo administered subcutaneously starting from the end of the first week of life. During rHuEpo therapy both groups received vitamin E (60 mg), calcium folinate (0.35 mg), and

Table 1

PRENATAL DATA OF NEONATES TREATED WITH PROTOCOL A

Pz	Previous pregnancies	N°IUT	GA(weeks) at IUT	Mode of delivery
1	1 Term, normal	4	26,30,32,33	CS
2	1 Term, normal 2 Spontaneous abortion 1 Preterm, died of isoimmunization	5	20,23,28,32,33	CS
3	1 Term, normal	6	23,27,29,30,33,35	VD
4	1 Term, normal 7 Spontaneous abortion	1	35	VD
5	1 Preterm, died of isoimmunization 1 Preterm, stillborn, isoimmunized	9	15,16,18,19,20,2124,25,27	CS
6	1 Term, normal 1 Spontaneous abortion	6	28,29,30,3133,34	CS

Table 2

PRENATAL DATA OF NEONATES TREATED WITH PROTOCOL B

7	1 Term, Prom 1 Preterm , Iso-Rh	1	31	CS
8	1 PretermIso-Rh 1 Term, Iso-Rh	2	20, 21	VD
9	2 Term, normal 1 Preterm, normal	4	21,25,28,30	CS
10	1 Voluntary termination of pregnancy 1 Early spontaneous abortion	3	21,31,32	CS
11	1 Term, normal 1 Preterm,,exitus at second day of life	1	24	CS
12	1 Preterm, normal 4 Spontaneous abortioni	4	20,23,29,31	CS
13	1 Voluntary termination of pregnancy,no prophylaxis 1 Preterm, Iso-Rh	3	23,24,27	CS
14	2 Term, normal	4	27,27,2728	CS

Table 3

NEONATAL DATA OF NEONATES TREATED WITH PROTOCOL A

Patient	GA (weeks)	BW(g)	SGA AGA	Sex	Apgar Score 1-5 min.	Ht at birth (%)	EXT (n°)	T before rHuEPO
1	38	3360	AGA	M	8 e 9	37	1	1
2	35	2300	AGA	F	3(INT) e 8	29	3	1
3	36	2420	AGA	M	6 e 7	45	NO	1
4	38	3060	AGA	F	9 e 10	41	NO	1
5	28	980	AGA	F	INT e INT	32	1	3
6	35	2990	AGA	M	8 e 9	35	NO	1

Table 4

NEONATAL DATA OF NEONATES TREATED WITH PROTOCOL B

Patient	GA (weeks)	BW(g)	SGA AGA	Sex	Apgar Score 1-5 min.	Ht at birth (%)	EXT (n°)	T before rHuEPO
7	31	1900	AGA	F	2(INT) e 5(INT)	44	NO	2
8	31	1800	SGA	F	9 e 10	54	1	NO
9	31	2200	AGA	M	8 e 9	30	1	1
10	32	2080	AGA	M	8 e 9	32	3	NO
11	35	2530	AGA	F	7 e 8	38	1	1
12	33	1220	SGA	F	7 e 8	41,2	NO	NO
13	28	770	SGA	M	7 e 8	25	1	NO
14	28	1870	AGA	M	5 e 7	36	3	2

iron maltose (20 mg/kg) administered intramuscularly on a weekly basis. The two groups were treated during a variable length of time, depending on the neonate's clinical response to the therapy. Each neonate was subjected to complete blood cell and reticulocyte count every week. Criteria for suspending the treatment were persistent thrombocytosis (platelet count > 600.000/mm^3) and severe persistent neutropenia (neutrophil count < 1000/mm^3). During the treatment, arterial blood pressure was measured daily to rule out the possible onset of hypertension.

Administration of PFRCs transfusion was defined as a failed rHuEpo therapy.

This need was established following the customary protocol for PFRC transfusion of the Neonatal Division of the University Polyclinic Agostino Gemelli that provides PFRCs transfusion when hematocrit <33% in neonates who are 28-day-old and/or are clinically instable or hematocrit <24% in neonates who are older than 28 days and/or are clinically stable, or have a reticulocyte count <2%.

Continuous variable data are reported as mean±SD. Categorical variables are reported as number (%). Group comparisons were conducted using a t-test or Mann-Whitney U test for continuous variables, and a Fisher exact test for categorical variables. Statistical analyses were conducted using Graphpad Prism 4.03 software for Windows.

The rHuEpo treatment was approved by the Ethical Committee of the Catholic University of the Sacred Heart of Rome, and was conducted upon receiving an informed consent from the parents of each patient.

Results

14 neonates were enrolled in the study. All babies received one or more IUT. The average number of IUTs was 5.1±2.6 per neonate treated according to protocol A and 2.7±1.2 per neonate treated according to protocol B (p<0.042).

Tables 1,2,3 and 4 report the prenatal and postnatal data for all of the enrolled neonates.

Mean gestational age at delivery was 35±4 weeks and mean birth weight was 2518±854.2 g for neonates treated according to protocol A: all were classified as appropriate for gestational age (AGA). Mean gestational age at delivery was 31,6±3 weeks and mean birth weight was 1796±558,5 g for neonates treated according to protocol B, 37.5% were classified as small for gestational age (SGA). There were no statistically significant differences for these characteristics in these two groups.

At birth, the mean hematocrit level was similar in these two groups (36.5±6% vs 37.5±9%). Three neonates of group A (50%) and six neonates of group B (75%) needed at least one EXT: 4 of them (n=2, 5,10, and 21) to treat early anemia, the others to treat hyperbilirubinemia. All were treated with phototherapy.

On average, rHuEpo therapy began at 16,5±2 days of life for neonates of protocol A and at 10,2±3 days of life for neonates of protocol B (p = 0,0047). The treatment lasted 33,6±21 days for neonates of protocol A and at 29,6±13 days of life for neonates of protocol B, this difference was not statistically significant.

Age at start of rHuEPO days	Days of treatmente with rHuEPO	T during rHuEPO (n°)	T after rHuEPO (n°)	Neonatal clinical course
19	38	NO	NO	Ventricular septal defect
16	32	1	1	Biventricular dyskinesia, tricuspid failure, pericardic fluid; Hepatosplenomegaly, cholestasis; Respiratory distress syndrome
14	21	1	NO	Congenital clubfoot. Inguinal hernia, dislocation of the hip
20	15	NO	NO	/
16	74	NO	3	Chronic lung disease, Sepsis, Retinopaty of prematurity, Atrial septal defect, patent ductus arteriosus
14	22	NO	2	Urinary tract infection

Age at start of rHuEPO days	Days of treatmente with rHuEPO	T during rHuEPO (n°)	T after rHuEPO (n°)	Neonatal clinical course
14	34	NO	NO	Pneumonia, Sepsis (E.Coli), Patent for a men ovale
7	23	NO	NO	Sepsis (E.Coli)
15	21	NO	NO	Respiratory distress syndrome type II
9	36	NO	NO	Sepsis, Patent fo a men ovale
11	16	NO	NO	Pulmonary thromboembolism, Respiratory distress syndrome, Baby bronze syndrome
10	16	1	1	Transitory myocardial ischemia, Lissencephaly, Retinopaty of prematurity
8	57	1	NO	Retinopaty of prematurity stage II zone II, Chronic lung disease, Phlebitis, Intra ventricular hemorragy grade I, Cardiac dyskinesia, Patent fo a men ovale
9	34	NO	NO	Retinopaty of prematurity, Chronic lung disease, intra ventricular hemorragy grade II, Pneumonia

All neonates of protocol A and fifty percent of neonates of protocol B needed one or more transfusions before rHuEpo therapy; 2 (33,3%) neonates of protocol A and 2 (25%) neonates of protocol B needed transfusion during the treatment; 3 (50%) of protocol A and 1 (12,5%) of protocol B needed transfusion after the end of treatment.

The 66,6% of protocol A needed one or more transfusions during or after the treatment, the 25% of neonates of protocol B needed one or more transfusion during or after the subministration of rHuEpo.

The neonates' average values of hematocrit, platelet, and neutrophil counted before and after 21 days of rHuEpo therapy did not differ significantly.

The hematocrit values in the protocol A group decreased slowly from the beginning to the end of the treatment (32,5% vs 25,2%), whereas the hematocrit value in protocol B group remained almost stable (38,7% vs 42,8%). The mean numbers of platelets remained stable in both groups while neutrophils increased in protocol A group and decreased in protocol B ($p<0,05$). Reticulocyte count increased during treatment in both groups, although only in protocol B group it was statistically significative ($p<0,05$). (Table 5)

The neonates who received the highest number of IUTs (9) received more PFRC transfusions (3) after the rHuEpo treatment and the longest duration of rHuEpo treatment (74 days).

Comparison of the considered parameters between two groups of infants did not reveal statistically significant differences.

Conclusions

Ohls et al. were the first to use rHuEpo in the treatment of late hyporegenerative anemia in 2 men-Rh patients.[6] Subsequently, there have been 3 other studies which have described the use of rHuEpo therapy.[8,15,16] Only one of them was a controlled-case study: it was conducted on 20 neonates (10 per group) which reported that treatment with rHuEpo reduced the need for transfusions with PFRCs in the treatment of late anemia caused by Rh-isoimmunization.[16]

In studies published to date, very different therapeutic protocols with rHuEpo have been used. The studies differ depending on treatment start time, and the use of iron, vitamin E, and folic acid supplements. Recently, few studies on isolated cases seem to be in agreement on recommending an earlier start of the treatment (within the first week of life), an higher dose of rHuEpo (from 200 U/kg 3 times a week to 870 U/kg/d)[17] and a longer duration treatment (from 3 to 4 wk to 5 to 6 wk).[11]

On this basis we compared the effects of erythropoietin therapy in two groups of infants who underwent two different treatment protocols for the prevention of anemia related to Rh-isoimmunization. No statistically significant differences between the two groups are evident on the parameters we considered for evaluating the effectiveness of erythropoietin in the prevention of late anemia.

Both groups of infants present an increase of reticulocyte count and hematocrit with the need to resort to PFRCs transfusions during or

Table 5

COMPARISON OF HT, RET, PLT, NEU BETWEEN PROTOCOL A AND B BEFORE RHUEPO THERAPY, AND AT THE END OF THE FIRST, SECOND AND THIRD WEEK OF TREATMENT.

	Ht%		Ret%		Plt10^9/l		Neu 10^9/l	
	Prot A	Prot B	Prot A	Prot B	Prot A	Prot B	Prot A	Prot B
Before	32,55	38,78	0,3	0,16	233	202,6	15,02	7,1
End of the first week	31,45	36,69	3,4	0,3	252,2	309	18,1	5,8
End of the second week	32,73	33,02	5,1	4,9	302,8	291,4	8,3	4,4
End of the third week	25,15	42,85	2,96	5,32	232	182,3	26,88	2,3

after treatment with erythropoietin. A major limitation of this work is the presence of statistically significant difference in the number of IUT performed by two groups of infants ($p<0.05$).

The effectiveness of treatment with rHuEPO is negatively affected by a larger number of IUT made. Indeed, the baby who received more IUT needed also more blood transfusions.

Moreover newborns who underwent a large number of IUT showed a significant bone marrow depression with anemia that requires an additional number of transfusions during the neonatal life.[18]

The cause of the findings is that IUTs performed with red blood cells containing hemoglobin A (that releases oxygen to the tissues better than hemoglobin F) have led to a significant reduction of the hypoxic stimulus on bone marrow. This has caused a reduced production of endogenous erythropoietin by the fetal liver and kidney. Therefore the infants who received a greater number of IUT have a depressed bone marrow and are more likely to not respond to rHuEPO.

In this study we have found out that the 66,6% of protocol A needed one or more transfusion during or after the treatment, while the 25% of neonates of protocol B needed one or more transfusion during or after the subministration of rHuEpo.

This seems to show a lower effectiveness of erythropoietin therapy in infants who had been submitted to Protocol A. The greatest number of transfusions received may depend significantly from the greater number of IUT received.

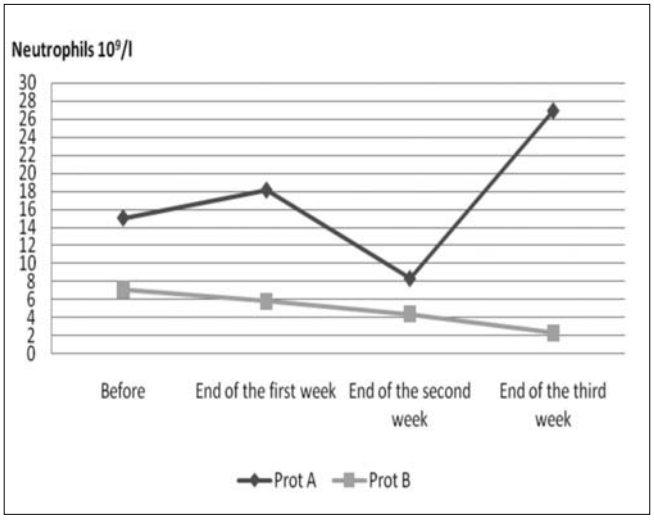

Figure 1.
Trend of Neutrophils during treatment

The lack of statistically significant differences in the number of postnatal EXT and ET who have been subjected infants of both groups and duration of treatment with erythropoietin highlights broadly similar efficacy of the two protocols.

As reported in previous studies, no infant has shown side effects.[10]

Infants who have undergone to Protocol A present reduction of neutrophils mean value by the end of the second week of treatment. After the second week, the neutrophils mean value begin to increase possibly because an increased bone marrow function. In infants subjected to Protocol B, instead, the neuthrophils count shows a constant declining trend reaching values also significantly lower than the original, even if remaining above the cut off necessary to define neutropenia (Figure 1). This could be possibly due to the effect of higher dose of erythropoietin administered to these infants that could have hampered a recovery production of white cells by the bone marrow.

Erythropoietin is an excellent therapeutic aid for the prevention anemia due to the late Rh-isoimmunization. It allows to reduce the need of transfusion therapy with PFCRs.

Our results suggest a similar efficacy between the two treatment protocols. We can say that it is not necessary to increase doses of erythropoietin for effective prevention of late onset anemia.

Further studies are needed to better define the protocol profile for which there is still no international consensus.

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