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Bilirubin and ascorbate antioxidant activity in neonatal plasma

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Abstract

Extremely low birth weight premature infants have been known for many years to have limited antioxidant protective capacity, especially with reference to those antioxidant components which do not cross the placenta until the third trimester of gestation. In this study the total antioxidant activity and the concentrations of individual antioxidants in plasma from premature neonates (27 ± 2 weeks gestation) compared to term babies (38–41 weeks gestation) have been examined. The results show elevated levels of ascorbate at birth in the plasma of premature neonates compared with those of term babies, but the total plasma antioxidant status of the premature babies is significantly lower than that of term babies. At 5 days post-partum the ascorbate levels are within the normal adult range and plasma bilirubin levels are considerably enhanced in both groups, while the total plasma antioxidant status of the premature neonates has increased. Analysis of the relationship between the total plasma antioxidant activity and the bilirubin concentration show a direct, highly significant correlation for the term group, $r^2 = 0.774$, consistent with significance of bilirubin as a plasma antioxidant.

Key words: Premature neonate; Total antioxidant activity; Bilirubin; Ascorbate

1. Introduction

Of the range of antioxidants in the human body, those located intracellularly are appropriate for dealing with aberrant generation of free radicals, whereas those placed extracellularly are more appropriate for intercepting propagating peroxidative mechanisms and for binding metal ions and delocalised haem proteins (reviewed in [1]). The antioxidant defences of human plasma include ascorbate [2], protein thiols [3], bilirubin [4], urate [5], and α-tocopherol [6], as well as the proteins involved in iron removal, namely, caeruloplasmin [7] and transferrin [1]. Oxidative stress is defined as a disturbance of the pro-oxidant/antioxidant balance in favour of the former [8]. Previous studies have shown that the total plasma antioxidant activity is decreased, compared to normal levels, in premature neonates [9], in smokers and asthmatics [10].

When, shortly after birth, premature infants require prolonged mechanical ventilatory support with high levels of oxygen therapy, the barotrauma, in combination with immature development of the lung antioxidant enzyme system, contributes towards the development of bronchopulmonary dysplasia or chronic lung disease of the newborn.

Extremely low birth weight (ELBW = ≥1000 g, ≥28 weeks gestation) premature infants have been known for many years to have limited antioxidant protective capacity. In particular, their vitamin E, β-carotene, caeruloplamin, α-antiproteinase, thiol-containing amino acid levels have all been shown to be deficient [11–14], mainly because these components do not cross the placenta until the third trimester of gestation. In addition, deficiencies in the trace metals selenium, copper and zinc, essential components of the antioxidant enzymes glutathione peroxidase and superoxide dismutase, have been reported [15].

In this study we have examined the total antioxidant activity and the concentrations of individual antioxidants of plasma from premature neonates (27 ± 2 weeks gestation) compared with those of term babies. The results show that premature neonates under the conditions studied have considerably higher ascorbate levels at birth compared to term babies, but their total plasma antioxidant status is significantly lower. The data also show that at 5 days post-partum the levels of the total antioxidant activity and ascorbate normalise and that an important contributing factor to the former is the bilirubin concentration in plasma.

2. Materials and methods

Ethical permission was obtained from the Ethics Committee of St. Thomas's Hospital.

The study group consisted of 31 preterm infants and 16 term infants who were delivered in the maternity unit at St. Thomas's Hospital. The pre-term infants had a gestational age of between 24 and 33 weeks with birth weight ranging from 446–1800 g. The pre-term infants were all admitted to the neonatal intensive care unit at St. Thomas's Hospital. The term infants had a gestational age between 37 and 41 weeks and their birth weights were between 2200 and 3920 g.

Umbilical cord blood samples were obtained from 16 term babies and 31 pre-term babies within 5 min after the delivery of the placenta. Blood was also collected from each baby on day 5.

Blood was gently withdrawn into heparinised tubes and immediately

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centrifuged (1000 × g, 15 min). The plasma was separated. 200 μl of plasma was added to 0.8 ml of 10% trichloroacetic acid, to obtain a protein-free extract for ascorbate analysis, and stored at −70°C. Other portions of the plasma were retained for total antioxidant activity, urate, albumin, ascorbate, α-tocopherol and stored at −20°C.

Total antioxidant activity was measured by the ABTS method on a Cobas Bio centrifugal analyser [9]. This method uses a spectrophotometric end point and commonly available laboratory reagents; the extent of quenching by antioxidants of the ABTS radical cation generated from interaction with activated myoglobin at 734 nm is measured. Uric acid and albumin were also measured by the Cobas Bio centrifugal analyser methods (uricase/4 aminophenazone and BCP). Plasma α-tocopherol was determined by normal-phase HPLC [16]. Total plasma ascorbate was determined by spectrophotometric analysis using 2,4 dinitrophenylhydrazine [17] and bilirubin according to Fog and Bakken [18].

3. Results

The characteristics of the premature babies are tabulated in Table 1. The correlation between birth weight and total antioxidant activity is shown in Fig. 1 ($r^2 = 0.43$) and confirms the previous proposition of a relationship between them [19].

Measurement of the levels of the major antioxidants in the plasma of premature neonates compared with term neonates (Table 2) shows that the two groups have

Table 1
Characteristics of premature neonates

Baby	Birth weight	Sex	Phototherapy	Gestation /40
TAL 1	1.45	M	Yes (days 3,4,5,6)	29
HAR 2	0.700	M		24^{+3}
AND 3	0.942	F	Yes (days 4,5,6,7)	27
SIM 4	1.40	F	No	29
CRA 5	1.80	F	No	31^{+5}
INN 6	0.730	F	Yes (days 3,4)	29
ECC 7	0.774	F	Yes (days 2,3,4,5)	25^{+2}
AMO 8	1.73	F	Yes (days 3,4,5)	32^{+5}
CUM 9	1.24	F	No	28
OLU 10	0.962	M	No	29^{+1}
FRE 11	0.446	F	No	28^{+4}
CRA 12	0.660	M	No	24^{+5}
SMI 13	1.45	F	No	30
PAT 14	0.952	F	No	28^{+3}
COC 15	1.18	M	Yes (days 4,5)	28^{+4}
PER 16	1.08	F	No	28^{+2}
MON 17	0.580	M	No	26^{+2}
MOO 18	1.5	M	Yes (days 3,4)	29^{+4}
WHI 19	0.926	F	Yes (days 2,3,4,5)	27^{+5}
BAC I 20	1.67	M	No	33^{+4}
BAC II 21	1.80	M	No	33^{+4}
THO 22	1.47	F	No	29
HAL 23	0.970	F	No	26
PAD 24	1.40	F	No	30^{+5}
JAC I 25	0.958	F	Yes (days 3,4)	26^{+5}
JAC II 26	0.990	F	Yes (days 5)	26^{+5}
MAR 27	1.38	M	No	28^{+3}
JAC 28	0.936	M	No	28^{+3}
JER 29	0.980	M	Yes (days 4,5)	26^{+2}
WAL 30	1.028	F	Yes (days 3,4,5)	26^{+5}
BAL 31	0.840	F	No	27^{+1}

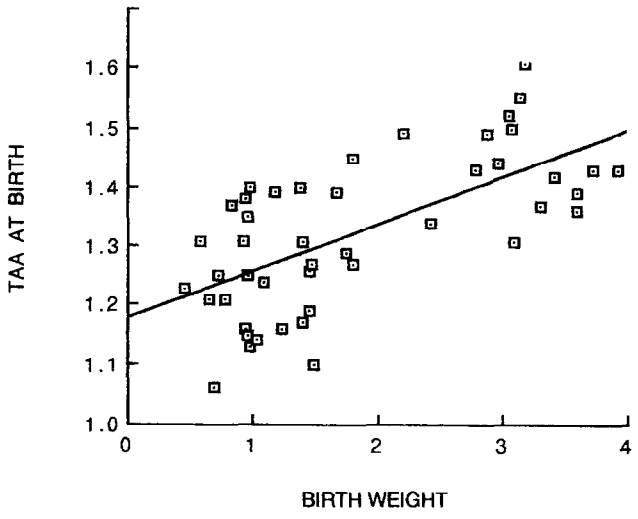

Fig. 1. Correlation between total plasma antioxidant activity at birth and birth weight in term and pre-term neonates ($r^2 = 0.434$).

no significant differences with respect to bilirubin and α-tocopherol at birth but that the latter is very much lower than the adult range. In contrast, the levels of albumin and urate are significantly lower in the samples from the premature babies, although the urate levels in both groups are within the normal reference interval; this only applies to the term babies for albumin.

Ascorbate levels are significantly higher in the premature babies at birth than in the term, and the values for term babies lie beyond the upper limit of the adult reference interval. Although, enhanced ascorbate levels in term babies has previously been demonstrated, no differences were revealed with premature babies at 32 ± 3 weeks [20].

At 5 days post-partum, while the albumin levels are not changed significantly in either group, the urate levels have significantly declined in the term babies but are still within the normal range. The ascorbate levels in both groups decreased to values close to the midpoint of the normal adult range and the α-tocopherol levels progressively increase as expected. Hyperbilirubinaemia is apparent in both groups at 5 days.

The total plasma antioxidant activities of the pre-term babies (27 ± 2 weeks) are consistent with our previous report on a smaller population, i.e. significantly depressed (1.25 ± 0.09, $n = 28$) compared to term babies (1.44 ± 0.08, $n = 16$) ($P < 0.005$) whose levels are akin to the normal adult range (1.46 ± 0.14, $n = 312$) [9]. After 5 days the mean value of total antioxidant activity had risen to values not significantly different from those of the term babies (pre-term, 1.45 ± 0.08, $n = 28$; term, 1.49 ± 0.15, $n = 15$).

In order to assess the relative importance of the plasma antioxidant profile in relation to the total antioxidant activity, the concentration of each of the antioxidants measured has been related to its overall antioxi-

Table 2
Plasma antioxidant levels (μM) of premature and term babies

	Values at birth		Values at day 5		Normal adult
	Premature	Term	Premature	Term	
Albumin	380 ± 83 [22]	482 ± 64 [14] $P < 0.005$	370 ± 80 [19]	530 [2]	535–760 midpt 640
Uric acid	267 ± 107 [24]	413 ± 103 [15] $P < 0.005$	196 ± 69 [27]	191 ± 84 [14]	180–420 midpt 300
Ascorbate	164 ± 60 [27]	123 ± 28 [16] $P < 0.005$	65 ± 25 [28]	84 ± 18 [16] $P < 0.05$	34–111 midpt 73
α-Tocopherol	6.8 ± 4.4 [10]	4.4 ± 1.4 [10]	10 ± 5.6 [23]	13.7 ± 4 [8]	14–44 midpt 29
Bilirubin	23 ± 10 [26]	20 ± 10 [16]	67 ± 47 [23]	126 ± 92 [16]	<20 midpt 10

dant potential [9] and expressed as relative proportions to the sum of the contributions from albumin, urate, ascorbate, α-tocopherol and bilirubin (the major radical scavenging antioxidants in plasma). The results (Table 3) show that for the pre-term and term babies, the combined relative contributions of urate, ascorbate and bilirubin at birth are the same, but the higher relative ascorbate and lower relative urate in the former are pronounced.

4. Discussion

Other workers [20] have measured the concentration of various plasma antioxidants of babies at 32 weeks gestation compared with term neonates and adults; their results revealed no significant differences between 32 ± 3 weeks pre-term and term neonates, but the ascorbate, vitamin E and bilirubin were all significantly different from adult levels.

The study described here shows elevated plasma ascorbate levels in the pre-term babies at birth compared to term. This may be a marker of the increased prematurity of these neonates and might be partly a compensatory mechanism for the relatively lower proportions of urate (Table 2), although not adequate enough to compensate for the lowered total antioxidant activity. Expressing the data as relative levels in relation to the major antioxidants of human plasma emphasises the contribution of bilirubin to the antioxidant potential at day 5 for both the term and the pre-term infants.

It is significant to note here that for the term babies a clear correlation is demonstrable (Fig. 2) between the total antioxidant activity and the bilirubin levels at day 5 ($r^2 = 0.774$) showing that the bilirubin concentration has a relatively greater impact on the total antioxidant activity. This demonstration of the importance of bilirubin as an antioxidant strengthens the proposal [21] that moderately increased plasma bilirubin levels may be positively favourable to infants under oxidative stress. For the premature babies the correlation is apparently much less significant ($r^2 = 0.30$), but this may be confounded by the phototherapy treatment which would influence the bilirubin levels.

The comparative data of relative levels of antioxidants suggest that the overall deficiency in the total plasma antioxidant capacities of the pre-term compared with term babies may stem from the contributions from the 'masked' oxidative stress factors which might include the iron status and the iron-binding proteins, or phagocytic activity. In the context of the iron status, several studies have demonstrated the presence of 'available' iron in the plasma of pre-term and term neonates [22–24], although the former will be more pronounced due to several factors, including transfusion. It has been proposed by others that iron-induced oxidative stress may play a role in the pathogenesis of oxygen radical diseases of prematurity [25]. Much work has also focussed on the iron status of the newborn and the importance of transferrin and caeruloplasmin levels in the context of antioxidant

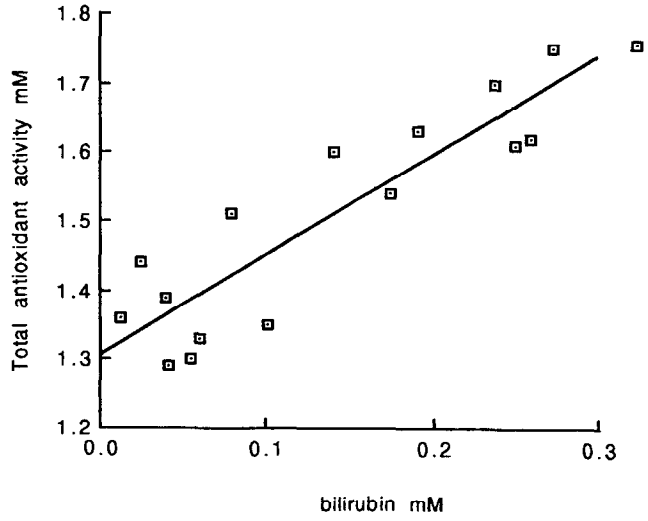

Fig. 2. Correlation between total plasma antioxidant activity (mM) and plasma bilirubin concentrations (mM) in term neonates, day 5 ($r^2 = 0.774$).

Table 3
Comparative relative proportions of plasma antioxidants between premature and term neonates expressed as a percentage of the total antioxidants

	Albumin	Urate	Ascorbate	α-Tocopherol	Bilirubin
TEAC value	0.63	1.02	0.99	0.97	1.5
Reference level [9]	49	37.5	9.5	3	1
Term at birth	34.5	47.8	13.8	0.5	3.4
Term - day 5	41	24.1	10.2	1.6	23
Prem at birth	33.5	38.1	22.7	0.9	4.8
Prem - day 5	30.8	26.4	8.5	1.2	33.1

activity [25]. However, exchange transfusion has been shown to have no influence on the TRAP value of plasma [26] in a gorup of new borns with rhesus haemolytic disease despite decreases in vitamin C and bilirubin, even though iron and ferritin levels declined and the caeruloplasmin and transferrin levels and the iron-binding capacity of the plasma increased.

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