Volume 303, number 2,3, 210–212 FEBS 11092 June 1992

Bleomycin-detectable iron in the plasma of premature and full-term neonates

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Received 11 March 1992; revised version received 13 April 1992

The bleomycin assay measures non-transferrin-bound iron, able to catalyze free radical reactions, in human plasma. No bleomycin-detectable iron is present in plasma from healthy adults. However, plasma from 3/15 premature babies was positive in this assay. Plasma from 52 apparently-healthy term babies was analyzed and 11 were positive in the bleomycin assay. Hence not only some premature but also some full-term apparently-healthy babies may be at risk of severe oxidative damage.

Neonate; Bleomycin; Iron overload; Iron metabolism; Transferrin; Free radical

1. INTRODUCTION

Oxygen-derived species such as superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) are continuously generated in the human body and may sometimes serve useful physiological roles (reviewed in [1]). Danger can arise when these species are brought into contact with 'free' transition metal ions. In particular, 'catalytic' iron ions can convert O_2^- and H_2O_2 into highly-reactive hydroxyl radicals and other damaging species. Iron can also accelerate the free radical chain reaction of lipid peroxidation [1,2]. Thus the antioxidant defences of the human body include not only enzymes that scavenge oxygen-derived species (superoxide dismutases, catalases, glutathione peroxidases), and inhibitors of lipid peroxidation (α-tocopherol), but also proteins, such as transferrin and lactoferrin, that sequester iron in 'safe' forms that will not catalyze free radical reactions and are generally unavailable to most species of bacteria [1,3–6]. Thus adult humans have only about 20–30% iron loading of plasma transferrin, and their plasma does not contain any iron ions available to catalyze free radical reactions [7].

Although these conclusions about human iron metabolism are well-established for adults, iron biochemistry in neonates may be different [8,9]. There are several reports that neonates, particularly pre-term, have compromised antioxidant defences, such as low activities of antioxidant enzymes [10] and low concentrations of tocopherols [11]. Thus it has been suggested that free radical activity is responsible for lung damage (e.g. bronchopulmonary dysplasia) in pre-term neonates [10,12] and may correlate with the chances of poor outcome [13]. Several reports also suggest that 'free' iron may be more available in pre-term neonates [8,9,14]. For example, in a study of rhesus haemolytic disease, Berger et al. [15] found that cord blood of babies with this disease had lower levels of iron-binding capacity and plasma ascorbic acid. Holton et al. [16] found that blood transfusions in the pre-term neonate were associated with a rise in transferrin saturation and, in some cases, transferrin was completely saturated. None of these authors measured non-transferrin-bound plasma iron directly, however.

Iron 'catalytic' for free radical reactions can be measured in plasma by the bleomycin assay introduced by Gutteridge et al. [7,17]. The 'free' iron ions are chelated by bleomycin and used to catalyze free radical damage to DNA, measured by a colorimetric reaction. The bleomycin assay can be applied directly to plasma samples and differences in the levels of antioxidants present do not affect it [7,17]. For example, the assay has been used to follow the progress of patients with iron overload consequent upon idiopathic haemochromatosis [18], to demonstrate iron overload caused by chemotherapy of patients with acute myeloid leukaemia [19] and to study recovery of normal iron metabolism after liver transplantation of patients with fulminant hepatic failure [20].

In the present paper, we have used the bleomycin assay to investigate directly previous suggestions [15,16] that the plasma of pre-term neonates may contain non-

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transferrin-bound iron. Plasma from healthy term neonates was used as a control: this control has not been carried out in previous studies, and the results were surprising.

2. SUBJECTS AND METHODS

The project was approved by the Hospital Ethical Committees. Two groups of babies were studied: 15 neonates admitted to the Special Care Baby Unit (SCBU) during the first 5 days of life (0.80–3.51 kg; 25–43 weeks gestation) and 52 apparently-normal term controls at birth (not requiring admission to SCBU and not receiving any blood transfusion).

Neonates on the SCBU were sampled only at times when blood was being drawn for clinical purposes. Only small blood samples (<1 ml) could be taken due to the low blood volume of neonates. Hence the study was designed as a random sample, most of the pre-term neonates being sampled only once.

In the second group (term babies), arterial blood was taken from the placenta within 2 min of delivery; APGAR score and arterial pH were also measured to give some indication of the degree of peripartum distress, if any. APGAR score is a simple clinical assessment of well-being based on respiration, heart rate, colour, muscle tone and reflex response. The maximum score is 10.

Bleomycin-detectable iron was measured as described in [17], using iron-free plastic materials throughout. Blood was drawn into heparinized containers, cells removed by centrifugation and the plasma immediately frozen to −70°C for transportation on dry-ice (total storage time <14 days, which has no effect on the parameters measured).

Transferrin iron saturation was measured by 6 M urea/polyacrylamide gel electrophoresis after pre-treatment of samples with Rivanol [21].

3. RESULTS

The results from the babies admitted to the SCBU and sampled during the first 5 days of life are given in Table I, in ascending order of birth weight. Three out of 15 showed the presence of bleomycin-detectable iron in their plasma. For two of these, plasma transferrin analysis showed 100% iron loading of this protein. Insufficient sample was available for this measurement to be performed on the third baby (Code JN). None of these babies had received blood transfusions. One other sample (CW) showed fully-saturated transferrin, but no bleomycin-detectable iron – this baby had received blood transfusion because of haemolysis. None of the neonates weighing >1.5 kg at birth (8 out of 15) were found to have bleomycin-detectable iron in their plasma.

Table II shows the results obtained by analyzing plasma from 27 term control babies. We were surprised to find that five showed bleomycin-detectable iron in their plasma. They did not appear any different from the other normal babies in terms of APGAR score at 5 min after birth, or arterial blood pH at birth. For two of these babies, transferrin was 100% saturated and for another two, saturation was >50%. For one baby (Code TI), saturation was <50%.

Because these results were so surprising, we obtained a further 25 term baby cord blood plasma samples. Six of these were positive in the bleomycin assay, with values of 2.1, 1.6, 0.9, 0.9, 0.7 and 0.65 μM. Blood pH values and APGAR scores were again normal. Plasma samples from healthy human adults analyzed with the same batch of reagents showed no bleomycin-detectable iron, as expected [7,17,18–20].

Table I

Plasma iron parameters of babies admitted to the special care baby unit

Key to clinical problem at the time of sampling: V = positive pressure ventilation; J = significant jaundice (requiring phototherapy); T = haemolysis requiring blood transfusion; A = recent asphyxia; NIL = no clinical problems other than low birth weight. *Key to transferrin saturation data:* – = not done (insufficient plasma sample available); U = not fully saturated; S = fully (100%) saturated.

	Samples first 5 days of life					
Patient code	Birth weight (kg)	Gestation (weeks)	Age (days)	Problem	Bleomycin iron (μmol/l)	Tf saturation
AC	0.80	25	1	V,J	0	–
OA	0.80	25	2	V	0	U
CHF	0.83	28	4	V	0	U
RC	1.25	28	3	V,J	2.1	S
SR	1.30	30	1	A	1.8	S
CW	1.43	32	3	V,T	0	S
JN	1.5	31	1	A,V	0.4	–
GL	1.78	32	3	V	0	U
GL	1.78	32	5	V	0	U
DW	1.82	30	2	V	0	–
MOB	2.04	34	0	NIL	0	U
MTB	2.07	34	0	NIL	0	U
SZ	2.3	34	0	NIL	0	U
MT	2.3	36	1	V	0	U
JM	3.1	38	4	V	0	–
FC	3.51	43	2	V	0	U

Table II

Plasma iron parameters of term control babies

Patient	Bleomycin iron (μmol/l)	Blood pH	APGAR score at 5 min	Transferrin saturation (%)
WY	0.85	7.329	9	100
BU	0.65	7.476	9	>50
AL	0.5	7.281	7	100
TI	0.4	7.307	9	<50
FR	0.35	7.305	9	>50
PR	0	7.316	9	>50
CU	0	7.260	9	>50
CR	0	7.188	9	>50
RA	0	7.408	9	>50
CC	0	7.104	9	>50
TL	0	7.340	10	>50
BL	0	7.321	9	>50
LU	0	7.345	9	>50
NE	0	7.449	9	>50
PH	0	7.307	8	>50
OS	0	7.316	9	<50
HU	0	7.252	9	<50
GI	0	7.327	9	<50
MO	0	7.308	9	<50
PA	0	7.338	9	<50
GR	0	7.268	4	<50
CH	0	7.309	9	<50
HA	0	7.355	5	<50
PE	0	7.154	8	<50
HR	0	7.344	10	<50
BA	0	7.311	10	<50
KA	0	7.195	9	<50

4. DISCUSSION

This paper reports the first *direct* demonstration of the presence of bleomycin-detectable iron in the plasma of neonates. Its presence in some pre-term neonates is consistent with earlier studies [8,9,15,16]. From our small random sampling during the first 5 days of life, the chance of finding bleomycin-detectable iron in a ventilated pre-term neonate weighing 1.5 kg or less appears to be of the order of 1-in-2 to 1-in-3.

However, a more surprising finding in our paper is the occurrence of bleomycin-detectable iron in the plasma of some apparently-healthy term neonates with good APGAR scores and approximately normal plasma pH. Our data suggest that iron metabolism in neonates may often be different from that in adults. The origin of the bleomycin-detectable iron is unknown. The presence of 'free' iron in the plasma of some neonates might mean that they are especially susceptible to damage by infection. First, the 'free' iron could facilitate bacterial growth [6]. Second, phagocytes activated by bacteria release O_2^- and H_2O_2 [22], which can form highly-damaging $OH^\cdot$ in the presence of catalytic iron ions. The babies might also be abnormally-susceptible to damaging side-effects from drugs whose metabolism generates O_2^- and H_2O_2 (many drugs are metabolized to free radical products [1]). In addition, several food additives might cause damaging free radical reactions in the presence of 'catalytic' iron [23]. Exposure of premature neonates to hyperoxia in the SCBU may also impose an oxidative stress that could be exacerbated by the presence of 'catalytic' iron [14,24].

It was also interesting to note the presence of bleomycin-detectable iron in some term babies, despite the coexistence of transferrin iron-binding capacity. Such an observation has previously been made in iron-overloaded patients after venesection treatment [18]. It may be that the iron exists as chelates that are poor donors of iron to transferrin [18]. In any case, further studies upon iron metabolism in premature and full-term babies in relation to their prospective health and survival seem urgently required.

Acknowledgements: B.H. thanks the British Heart Foundation and the Arthritis and Rheumatism Council for research support. We thank the neonatal nursing staff at West London Hospital and the midwives at St. Mary's Hospital Portsmouth for their assistance.

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