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Iron overload in paediatrics undergoing cardiopulmonary bypass

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Abstract

Pathological changes in iron status are known to occur during bypass and will be superimposed upon physiological abnormalities in iron distribution, characteristic of the neonatal period. We have sought to define the severity of iron overload in these patients. Plasma samples from 65 paediatric patients undergoing cardiopulmonary bypass (CPB) were analysed for non-haem iron, total iron binding capacity, transferrin and bleomycin-detectable iron. Patients were divided into four age groups for analysis. Within each age group, patients who were in iron overload at any time point were statistically compared to those who were not. The most significant changes in iron chemistry were seen in the plasma of neonates, with 25% in a state of plasma iron overload. 18.5% of infants and 14.3% of children at 1–5 years were also in iron overload at some time point during CPB. No children over 5 years, however, went into iron overload. Increased iron saturation of transferrin eliminates its ability to bind reactive forms of iron and to act as an antioxidant. When transferrin is fully saturated with iron, reactive forms of iron are present in the plasma which can stimulate iron-driven oxidative reactions. Our data suggest that paediatric patients are at greater risk of iron overload during CPB, and that some form of iron chelation therapy may be advantageous to decrease oxidative stress.

Keywords: Iron overload; Cardiopulmonary bypass; Antioxidant; Chelation; Transferrin; Neonate

1. Introduction

Iron is a transition metal widely used in nature to transfer electrons, transport oxygen and act catalytically at the active centre of oxidases, oxygenases and certain antioxidants (reviewed in [1]). The ability of iron to redox cycle and transfer electrons to molecular oxygen to form a variety of reactive oxygen species (ROS) has necessitated the evolution of ligands that decrease or prevent unwanted electron transfers. Thus, iron transporting proteins such as transferrin and lactoferrin, and iron storage proteins such as ferritin and haemosiderin, sequester iron in forms that minimise the transfer of electrons from iron to molecular oxygen [2,3].

Most cells contain a pool of low molecular mass iron (LMrFe) for the synthesis of iron-containing

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proteins and DNA, whereas most extracellular fluids normally do not. Normal human plasma, therefore, contains no detectable LMrFe because the iron binding protein transferrin is only around 30% loaded with iron retaining a considerable iron binding capacity. When, however, the release of chelatable iron exceeds the iron binding capacity of transferrin, LMrFe can be detected and measured in plasma, using techniques such as the bleomycin assay [4].

Plasma iron overload can be found in a variety of pathological conditions (reviewed in [5]) as well as physiologically in the neonate [6,7]. A high percentage of blood samples from term and pre-term neonates show full iron saturation of transferrin with the presence of LMrFe in the plasma [6,7] and some of this iron may be present in the highly reactive ferrous (Fe^{2+}) form [8]. Ferrous salts have the potential to transfer electrons directly to molecular oxygen to form ROS. Neonates have antioxidant defences profoundly different from adults and these do not appear to cope well with the additional generation of ROS [9,10]. When pro-oxidants, such as LMrFe, are increased or antioxidant protection diminished a situation of oxidative stress will ensue.

During adult cardiopulmonary bypass iron is released as a result of the extracorporeal circulation of blood [11], ischaemia–reperfusion [12] and blood cardioplegias [13]. A combination of these factors increases the iron saturation of transferrin in all adult bypass patients to over 50%. Of these some 19% will be in plasma iron overload with a fully iron-saturated transferrin [11,13]. These findings in adults prompted us to undertake a detailed study in neonates, infants and children undergoing cardiopulmonary bypass in whom pathological changes in iron chemistry due to bypass will be superimposed upon their physiological differences in iron metabolism.

2. Materials and methods

Bleomycin sulphate and calf thymus DNA were from the Sigma Chemical Co., Poole, Dorset, UK. Radial immunodiffusion plates for transferrin assays were obtained from Dade Behring Diagnostics, Milton Keynes, UK. All other chemicals were of the highest grades available from Fisher Scientific UK, Loughborough, Leics, UK.

2.1. Bypass blood samples

The study was approved by the Royal Brompton Clinical Research Ethics Committee, and parents of all subjects provided informed consent. Blood samples were collected into lithium heparin tubes and sent to the laboratory for immediate separation. Plasma was stored at −20°C until the time of analysis, which was no longer than 7 days after separation.

2.2. Bypass conditions

Cross clamp, bypass and arrest times are summarised in Table 1, together with mortality rates and the type of cardioplegia used. The following age groupings were made: Neonates, up to 1 month; Infants, >1 month to 1 year; Children >1 year to 5 years and Children >5 years to 10 years.

Table 1
Summary of clinical cardiopulmonary bypass surgery details

	Neonate	Infants	Children 1–5 years	Children 5–10 years
$N=$	12	26	21	6
Sex (male)	7	11	10	2
Cross clamp time (min)	92.3 ± 7.7 ($n=11$)	52.3 ± 4.1 ($n=25$)	53.2 ± 6.9 ($n=19$)	31.5 ± 5.4 ($n=6$)
Bypass time (min)	132.7 ± 11.2 ($n=11$)	83.7 ± 7.7 ($n=25$)	74.2 ± 8.8 ($n=19$)	45.3 ± 7.8 ($n=6$)
Arrest time (min)	23.2 ± 4.5 ($n=11$)	35.8 ± 9.1 ($n=6$)	0	0
Mortality ($n=$)	2	5	0	0
Cardioplegia				
Blood ($n=$)	3	12	8	2
Crystalloid ($n=$)	9	14	13	4

2.3. Bleomycin assay for LMrFe

Iron in plasma that is chelatable and redox active was measured using the bleomycin assay [4]. Briefly, the reaction mixture contained DNA (1 mg/ml), bleomycin (1.5 U/ml), Tris buffer (1 M pH 7.4), magnesium chloride (50 mM) and the plasma sample (20 μl). Ascorbic acid (7.5 mM) was added to start the reaction and the mixture was incubated at 37°C for 30 min. Any iron chelated from the plasma sample by bleomycin was reduced to the ferrous state by ascorbate. A ternary complex between iron, bleomycin and DNA in the presence of molecular oxygen formed oxo-iron species that degraded DNA and released malondialdehyde (MDA) from the deoxyribose sugar. MDA was measured spectrophotometrically ($A_{532\,nm}$) after reacting it with 2-thiobarbituric acid (TBA). To control for any TBA reactivity in the plasma samples not produced by the bleomycin–iron–DNA complex, a control was set up for each sample. All the same reagents were used in the sample control except chelex-treated water was substituted for bleomycin. The result of this reaction was then subtracted from the result obtained with bleomycin.

Adventitious iron in reagents was removed as previously described [4]. LMrFe was quantitated with reference to pure iron standards.

2.4. Total plasma non-haem iron and iron binding capacity

The total plasma non-haem iron and the iron binding capacity were measured using a Sigma kit assay based on the ferrozine spectrophotometric method.

2.5. Transferrin

Plasma transferrin was quantitated using radial immunodiffusion plates containing a polyclonal antibody to human transferrin and pure standards of human apotransferrin. The percentage saturation of transferrin with iron was derived from the measured iron binding capacity. This was found to be in close agreement with values calculated from the amount of transferrin present. Where appropriate, values are corrected to the plasma total protein value (assayed by the Lowry technique) to adjust for haemodilution inherent in bypass procedures.

2.6. Statistical analysis

Statistical comparisons were made using the Mann–Whitney *U*-test.

3. Results

3.1. Low molecular mass iron

Neonates, as a group, showed more severe abnormalities in their iron chemistry before and during cardiopulmonary bypass. One of the neonates (8% of the total) was in iron overload before bypass, and this iron overload was seen in all subsequent samples during and after bypass, although the values fell, presumably due to haemodilution (Table 2). Two other neonates became iron-overloaded by the end of bypass, representing 25% of the total in this group. Neonates that showed iron overload at any time point before, during or after bypass have been grouped as the 'bleomycin iron-positive' sub-group and statistically compared with those who did not show BLM-Fe at any time point (Table 2).

3.2. Transferrin

Levels of plasma transferrin pre-bypass in the neonates and infants were low when compared to normal healthy adult levels, but were within the normal range expected for normal neonates and infants. As bypass progressed, transferrin levels initially fell due to haemodilution but then slowly recovered to reach values somewhat lower than the pre-bypass levels (Table 2).

3.3. Non-haem iron and iron overload

The amount of non-haem iron in the plasma increased in all patient groups during bypass, although the neonates showed higher pre-bypass levels. Values appeared to fall initially, but increased towards the end of bypass (Table 2). An increased release of iron into the plasma accompanied by a falling level of transferrin resulted in the transferrin becoming

Table 2
Changes in plasma iron values of neonates (up to 1 month) undergoing cardiopulmonary bypass (CPB)

$N = 12$	Total non-haem iron (nmol/mg protein)	Total iron binding capacity (nmol/mg protein)	Transferrin (g/l)	% Saturation of transferrin	Bleomycin-detectable iron (BLM-Fe) (μmol/l)
Pre CPB					
BLM-Fe −ve	0.424 ± 0.04	0.822 ± 0.07	1.73 ± 0.16	53.3 ± 4.9	0
BLM-Fe +ve	0.465 ± 0.20	0.593 ± 0.08	1.36 ± 0.29	60.9 ± 23.6	4.99 (1 sample)
CPB on					
BLM-Fe −ve	0.359 ± 0.03	0.573 ± 0.06	0.80 ± 0.07	63.0 ± 4.8	0
BLM-Fe +ve	0.392 ± 0.11	0.618 ± 0.09	0.66 ± 0.04	69.6 ± 23.3	0.66 (1 sample)
Cross clamp off					
BLM-Fe −ve	0.411 ± 0.04	0.640 ± 0.07	0.99 ± 0.14	66.5 ± 5.4	0
BLM-Fe +ve	0.515 ± 0.11	0.527 ± 0.06	0.64 ± 0.05	92.6 ± 7.4	0.69 (1 sample)
CPB off					
BLM-Fe −ve	0.375 ± 0.04	0.650 ± 0.06	1.07 ± 0.14	58.4 ± 5.1	0
BLM-Fe +ve	0.590 ± 0.08*	0.510 ± 0.05	0.75 ± 0.09	98.5 ± 1.5*	0.47 ± 0.17(3 samples)
2 h post CPB					
BLM-Fe −ve	0.520 ± 0.05	0.748 ± 0.08	1.36 ± 0.12	69.6 ± 4.3	0
BLM-Fe +ve	0.597 ± 0.27	0.639 ± 0.12	1.32 ± 0.23	81.7 ± 18.4	0.73 (1 sample)

Plasma samples which contained non-transferrin bound LMrFe which was detectable using the bleomycin assay (BLM-Fe +ve) at any time point during CPB were compared with those which did not show the presence of non-transferrin bound iron (BLM-Fe −ve).
One neonate showed the presence of BLM-Fe before bypass (4.99 μmol/l), and such iron was present in the plasma of this patient at all time points during bypass. At the time point 'CPB off' two other neonates became iron overloaded. These three neonates have been grouped together and compared with the nine neonates who did not show iron overload at any time during CPB. Results are shown as mean ± S.E.M. $*P < 0.05$.

more loaded with iron, and decreasing the iron binding capacity of transferrin. The percentage saturation of transferrin rose in all patient groups during bypass and this was most noticeable in the 'bleomycin-positive' patients whose plasma transferrin iron loading significantly increased (Tables 2–5). Each individual patient showing bleomycin-detectable iron, at a given time point, had fully iron-loaded (100% saturated)

Table 3
Changes in plasma iron values of infants (1 month to 1 year) undergoing cardiopulmonary bypass (CPB)

$N = 26$	Total non-haem iron (nmol/mg protein)	Total iron binding capacity (nmol/mg protein)	Transferrin (g/l)	% Saturation of transferrin	Bleomycin-detectable iron (BLM-Fe) (μmol/l)
Pre CPB					
BLM-Fe −ve	0.393 ± 0.04	1.12 ± 0.06	2.15 ± 0.11	35.7 ± 3.0	0
BLM-Fe +ve	0.515 ± 0.05*	1.29 ± 0.13	2.09 ± 0.30	40.7 ± 2.8	0
CPB on					
BLM-Fe −ve	0.531 ± 0.05	0.962 ± 0.04	1.06 ± 0.07	51.3 ± 3.8	0
BLM-Fe +ve	0.559 ± 0.09	0.938 ± 0.25	1.14 ± 0.30	67.8 ± 13.0	0.348 ± 0.33 (2 samples)
Cross clamp off					
BLM-Fe −ve	0.428 ± 0.03	0.878 ± 0.04	1.23 ± 0.1	49.7 ± 3.3	0
BLM-Fe +ve	0.674 ± 0.23	1.066 ± 0.40	1.12 ± 0.5	65.3 ± 34.8	0.24 (1 sample)
CPB off					
BLM-Fe −ve	0.493 ± 0.04	0.966 ± 0.05	1.41 ± 0.13	51.9 ± 3.7	0
BLM-Fe +ve	0.887 ± 0.12*	0.885 ± 0.16	0.92 ± 0.19	97.6 ± 2.1*	0.623 ± 0.29 (5 samples)
2 h post CPB					
BLM-Fe −ve	0.571 ± 0.05	0.946 ± 0.08	1.83 ± 0.11	61.4 ± 4.7	0
BLM-Fe +ve	1.040 ± 0.06*	0.885 ± 0.08	1.51 ± 0.15	100 ± 0.03*	2.77 ± 2.07 (3 samples)

No infant showed the presence of BLM-Fe before bypass. However, five infants went into iron overload at various time points during CPB. Statistical comparisons are made as described in the footnote to Table 2.

Table 4
Changes in plasma iron values of children (1–5 years) undergoing cardiopulmonary bypass (CPB)

$N = 21$	Total non-haem iron (nmol/mg protein)	Total iron binding capacity (nmol/mg protein)	Transferrin (g/l)	% Saturation of transferrin	Bleomycin-detectable iron (BLM-Fe) (μmol/l)
Pre CPB					
BLM-Fe −ve	0.363 ± 0.02	1.050 ± 0.07	2.31 ± 0.12	36.3 ± 2.3	0
BLM-Fe +ve	0.394 ± 0.07	0.983 ± 0.11	2.12 ± 0.42	41.9 ± 11.0	0
CPB on					
BLM-Fe −ve	0.408 ± 0.02	0.933 ± 0.09	1.10 ± 0.09	49.3 ± 4.0	0
BLM-Fe +ve	0.594 ± 0.09*	0.833 ± 0.02	0.96 ± 0.23	71.7 ± 11.4	0
Cross clamp off					
BLM-Fe −ve	0.421 ± 0.02	0.871 ± 0.09	1.13 ± 0.1	54.7 ± 5.2	0
BLM-Fe +ve	0.609 ± 0.09*	0.732 ± 0.14	0.97 ± 0.3	79.7 ± 17.8	0.088 ± 0.07 (2 samples)
CPB off					
BLM-Fe −ve	0.443 ± 0.02	0.855 ± 0.06	1.32 ± 0.08	53.9 ± 3.3	0
BLM-Fe +ve	0.494 ± 0.13	0.759 ± 0.13	1.37 ± 0.30	61.0 ± 19.6	0.241 (1 sample)
2 h post CPB					
BLM-Fe −ve	0.520 ± 0.04	0.766 ± 0.03	1.63 ± 0.03	68.1 ± 4.4	0
BLM-Fe +ve	0.605 ± 0.13	0.834 ± 0.05	1.65 ± 0.14	72.8 ± 14.8	0.146 (1 sample)

None of the children (1–5 years) showed the presence of BLM-Fe before bypass. However, three of the children went into iron overload during the bypass procedure. See Table 2 for method of statistical analyses.

transferrin, although this is not always obvious from the mean values shown. All bypass patients who did not show the presence of bleomycin-detectable iron nevertheless increased the iron saturation of their transferrin to twice the normal level (60%) during the bypass procedure (Tables 2–5). 18.5% of infants showed iron overload during cardiopulmonary bypass whereas 14.3% of children 1–5 years, and no children above the age of 5 showed iron overload (Tables 3–5).

4. Discussion

Extracorporeal circulation of blood during cardiopulmonary bypass surgery exposes cells to non-physiological surfaces and shear stresses, which activate several regulatory enzyme cascades, activate neutrophils to release superoxide and hydrogen peroxide, and lyse red blood cells to release their contents including haemoglobin [14]. Hydrogen peroxide can react with haemoglobin to form a ferryl iron species and a protein radical [15], and if sufficient H_2O_2 is present the iron–protein complex of haemoglobin fragments to release LMrFe detectable by the bleomycin assay [15]. We speculate that the origin of this LMrFe seen during cardiopulmonary bypass surgery is from haemoglobin and not from ferritin, since levels of this protein on the whole do not increase during bypass surgery. Additionally changes in iron release are in parallel with an increase in thiols seen during cardiopulmonary bypass [13]. The source of these thiols is thought to be from haemolysis of red blood cells during surgery, which contain high levels of the thiol group containing molecule glutathione (approximately 800 μM per litre of blood). Hydrogen peroxide is formed by activated phagocytic cells, and also comes from biochemical reactions initiated during the period of ischaemia–reperfusion when the aortic cross clamp is released. Aberrant ATP catabolism during cardiopulmonary bypass ischaemia causes a significant increase in plasma hypoxanthine levels [16]. Hypoxanthine (a substrate for xanthine oxidase, an enzyme formed from xanthine dehydrogenase by oxidative or proteolytic modification) is converted to uric acid with the formation of superoxide radicals and H_2O_2.

The type of cardioplegia used in adult cardiopulmonary bypass has been shown to influence levels of both hypoxanthine [16] and LMrFe in the plasma [13]. In this study, neonates, infants, and children received either crystalloid or cold blood cardioplegia, and of those receiving blood cardioplegia 82% were in iron overload at some point during cardiopulmo-

Table 5
Changes in plasma iron values of children (5–10 years) undergoing cardiopulmonary bypass (CPB)

$N=6$	Total non-haem iron (nmol/mg protein)	Total iron binding capacity (nmol/mg protein)	Transferrin (g/l)	% Saturation of transferrin	Bleomycin-detectable iron (BLM-Fe) (μmol/l)
Pre CPB					
BLM-Fe −ve	0.398 ± 0.04	0.925 ± 0.06	2.06 ± 0.11	44.8 ± 6.8	0
BLM-Fe +ve	0	0	0	0	0
CPB on					
BLM-Fe −ve	0.501 ± 0.07	0.899 ± 0.11	1.28 ± 0.14	59.2 ± 9.0	0
BLM-Fe +ve	0	0	0	0	0
Cross clamp off					
BLM-Fe −ve	0.484 ± 0.09	0.843 ± 0.12	1.29 ± 0.16	63.2 ± 15.2	0
BLM-Fe +ve	0	0	0	0	0
CPB off					
BLM-Fe −ve	0.562 ± 0.08	0.966 ± 0.09	1.45 ± 0.12	61.3 ± 9.7	0
BLM-Fe +ve	0	0	0	0	0
2 h post CPB					
BLM-Fe −ve	0.513 ± 0.06	0.886 ± 0.08	1.77 ± 0.07	59.1 ± 6.3	0
BLM-Fe +ve	0	0	0	0	0

None of the children (5–10 years) showed the presence of BLM-Fe before or during the bypass procedure.

nary bypass. This observation suggests that blood cardioplegia may put paediatric patients at greater risk of oxidative stress. Overall, neonates showed a higher incidence of plasma iron overload than that seen in adults, whereas infants showed a pattern similar to that seen in adults randomised to receive all three cardioplegia regimens [13]. It could be argued that the higher incidence of iron overload in the neonate group could be attributed to the longer cross clamp and bypass times (see Table 1). However, we have previously shown differential iron release in an adult cardiopulmonary bypass population receiving different cardioplegia regimes who had comparable cross clamp and bypass times to each other [13]. Children in the age group 1–5 years showed a lower incidence of iron overload than adults, and children over 5 years showed no cases of iron overload, probably reflecting their higher plasma transferrin levels. Adult patients undergoing cardiopulmonary bypass have previously been shown to have low plasma transferrin levels, and to have decreased antioxidant protection against iron toxicity [17].

Levels of non-haem iron increased in the plasma of neonates, infants and children (who did not show bleomycin-detectable iron) during the bypass procedure, to cause increased loading of the transferrin with iron by up to 60%.

In adult cardiopulmonary bypass patients it is well established that mild lung injury is a common complication of surgery, and previously [18] we have demonstrated that during cardiopulmonary bypass, lung permeability correlates with the iron loading of transferrin. LMrFe is a potent catalyst for a variety of oxidative reactions, often involving free radicals (reviewed in [19]), as well as a virulence factor for microbial growth [20]. It is, therefore, important to control or remove LMrFe from the plasma. Data presented here support the postulate that neonates are at greater risk from iron overload during cardiopulmonary bypass, and suggest that some form of iron chelation therapy might be considered. For neonates, with a permeable blood–brain barrier, this may best be given in the form of a recombinant human transferrin or lactoferrin, whereas for children and adults, a low molecular mass iron chelator such as desferrioxamine may be more suitable.

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References

[1] B. Halliwell, J.M.C. Gutteridge, Methods Enzymol. 186 (1990) 1–85.
[2] M.J. O'Connell, B. Halliwell, C.P. Moorhouse, O.I. Aruoma, H. Baum, T.J.G. Peters, Biochem. J. 234 (1986) 727–731.
[3] J.M.C. Gutteridge, S.K. Paterson, A.W. Segal, B. Halliwell, Biochem. J. 199 (1981) 259–261.
[4] J.M.C. Gutteridge, Y. Hou, Free Radical Res. 2 (1986) 143–151.
[5] J.M.C. Gutteridge, in: E. Griffiths, J.J. Bullen (Eds.), Iron and Infection, Physiological and Clinical Aspects, 2nd edn., Wiley, Chichester, 1996.
[6] J.H.N. Lindeman, E. Houdkamp, E.G.W. Lentjes, B.J.H. Poorthuis, H.M. Berger, Free Radical Res. 16 (1992) 285–294.
[7] P.J. Evans, R. Evans, I.Z. Kovar, A.F. Holten, B. Halliwell, FEBS Lett. 303 (1992) 210–212.
[8] H.M. Berger, S. Mumby, J.M.C. Gutteridge, Free Radical Res. 22 (1994) 555–559.
[9] R. Bracci, in: J.G. Koppe (Ed.), Care, Concern and Cure in Perinatal Medicine, Parthenon Publishing Group, Lancs, 1993, pp. 379–389.
[10] J.H.N. Linderman, D. von Zoeren-Grobben, J. Schrijver, A.J. Speek, B.J.H. Poorthuis, H.M. Berger, Pediat. Res. 26 (1989) 20–24.
[11] J.R. Pepper, S. Mumby, J.M.C. Gutteridge, Free Radical Res. 21 (1994) 53–58.
[12] A. Voogd, W. Sluiter, J.F. Koster, Free Radical Biol. Med. 16 (1994) 453–458.
[13] J.R. Pepper, S. Mumby, J.M.C. Gutteridge, Ann. Thorac. Surg. 60 (1995) 1735–1740.
[14] N.E. Moat, T.W. Evans, G.J. Quinlan, J.M.C. Gutteridge, FEBS Lett. 328 (1993) 103–106.
[15] J.M.C. Gutteridge, FEBS Lett. 201 (1986) 291–295.
[16] G.J. Quinlan, S.T. Westerman, S. Mumby, J.R. Pepper, J.M.C. Gutteridge, J. Cardiovasc. Surg. 40 (1999) 65–69.
[17] J.R. Pepper, S. Mumby, J.M.C. Gutteridge, Free Radical Res. 21 (1994) 377–385.
[18] M. Messent, D.G. Sinclair, G.J. Quinlan, S. Mumby, J.M.C. Gutteridge, T.W. Evans, Crit. Care Med. 25 (1997) 425–429.
[19] B. Halliwell, J.M.C. Gutteridge, FEBS Lett. 307 (1992) 108–112.
[20] C.G. Ward, J.J. Bullen, H.J. Rogers, J. Trauma 41 (1996) 356–364.