Impaired bacteriological responses in babies after maternal iron dextran infusion

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SUMMARY The effect of a total dose infusion of iron dextran in pregnancy on 15 mothers and their babies was compared with 19 controls. The bacteriostatic effect and opsonising ability of the sera, of babies born to the treated mothers, were considerably impaired. This was associated with a significantly lower transferrin concentration in these mothers. Although these in vitro tests were not associated with an increase in overt infection during the perinatal period, they suggest the need for caution in the use of total dose infusions in pregnancy.

Recent reports by Becroft, Dix, and Farmer¹ and Barry and Reeve² have suggested that babies treated with iron dextran (Imferon) by injection, for prevention of anaemia, are more susceptible to infection by *Escherichia coli* (*E coli*). It was considered that a similar problem might possibly occur with babies born to mothers who had received a single total dose infusion (TDI) of iron dextran. A study was therefore implemented to examine the effects of maternal TDI on the haemoglobin (Hb), serum iron, and transferrin concentrations of mother and baby and also on in vitro tests of bacteriological competence of the baby.

Subjects and methods

A group of 15 mothers who had received a TDI and their babies were compared with 19 control pairs. All were caucasians, managed by the same obstetric teams and tested during a four month period. TDI was given for persistently low maternal Hb and serum iron concentrations, and failure to respond to oral iron supplements. Controls were selected to match for maternal parity, baby's sex and gestational age. All babies were term by dates and clinical assessment, although one in the treated group was light for dates. Consent for investigation was obtained from the mother, before or as soon as possible after labour began. Blood was taken from her at this time, for Hb, serum iron, and transferrin. Samples of cord blood were taken from all babies for the same tests. In addition 13 babies of the treated group and 17 of the control group had cord blood collected for the

bacteriological tests set out below.

At three to five days of age capillary blood was collected from all babies for a nitroblue tetrazolium test and stools taken for culture.

HAEMATOLOGY AND BIOCHEMISTRY

Haemoglobin concentrations were measured using a Coulter S counter. Serum iron concentrations were determined on a Technicon AA11 AutoAnalyzer using a modification of the method of Young and Hicks.³ The normal range for adult females is 5-30 $\mu\text{mol/l}$ (28-168 $\mu\text{g}/100\text{ ml}$), and for cord blood is 14-43 $\mu\text{mol/l}$ (78-240 $\mu\text{g}/100\text{ ml}$).⁴

Transferrin was measured using an automated immuno-precipitin method,⁵ with polyethylene glycol to accelerate the antigen-antibody reaction. The normal range for adults is 2.0-4.0 g/l and for cord blood is 1.47-2.12 g/l.⁴ Total iron binding capacity was calculated from the serum transferrin, by multiplying the figure by 1.25⁴ and the percentage saturation of transferrin was calculated from the concentrations of serum iron and total iron binding capacity.

BACTERIOLOGY

Stools from both groups of neonates were examined for pathogenic coliforms. Tests for *Salmonella* spp were carried out using selenite broth as an enrichment medium, followed by plating on Wilson and Blair, and deoxycholate citrate agar. Specimens for *Shigella* spp isolation were plated directly on deoxycholate citrate agar and for *E coli* directly on to 5% horse blood agar and MacConkeys medium. Potentially pathogenic *E coli* were tested using polyvalent antisera groups I, II, and III.

Cellular immunity

Bactericidal capacity of cord blood phagocytes against *E coli* National Collection of Type Cultures (NCTC) strain 9026 (group 026: k60 (Bb): H11) was studied using a method⁶ that determines the percentage of *E coli* killed by leucocytes during a two-hour incubation period.

Nitroblue tetrazolium tests were performed using the methods of Gifford and Malawista.^{7,8} Capillary blood phagocytes adherent to a glass surface, were incubated with nitroblue tetrazolium in the presence or absence of yeast particles (zymosan). The percentage of formazan-containing cells were counted microscopically in both "glass-adherent" and "zymosan-stimulated" preparations.

Humoral immunity

The opsonising properties of each test serum were compared by their effect in 10% and 2.5% final dilutions on the killing of *E coli* NCTC strain 9026 by normal washed neutrophils.

Bacteriostatic effect

The ability of each serum to support the growth of *E coli* NCTC strain 9026 was compared. The method previously described by Becroft *et al.*¹ requires a standard number of organisms (1×10^8 organisms/ml) in Hank's balanced salt solution containing 0.1% gelatine. To this was added previously heated

test sera (56°C for 1 h) to make a final dilution of sera to 1/2. Each incubation mixture was sampled at 30 min intervals during a five and a half hour incubation. The bacteria were counted by a plating technique and the numbers compared with the original count, to give a multiplication factor.

Statistical analysis was by Student's *t* test and testing of correlation coefficient *r*.

Results and discussion

The Hb, transferrin, and serum iron concentrations on the cord blood specimens were similar for both groups of babies (Table 1). There was no significant difference in the birth weight of the two groups of babies (treated (mean $\pm$ SD) 3.43 ± 0.6 kg; control 3.44 ± 0.4 kg). Mothers who had received Imferon injections had significantly lower transferrin concentrations than control groups ($p < 0.001$), but their serum iron, transferrin saturation and Hb results were similar (Table 1). In this study, no correlation was demonstrated between maternal and infant transferrin in either group.

The most significant findings were in the bacteriology studies (Table 2). The cellular response was not impaired, both test and control babies performing equally well. However, the differences in humoral responses were highly significant. Those babies exposed to iron dextran in utero were less able to

Table 1 Haemoglobin and iron status (means $\pm$ SD) of mothers and babies in maternal and cord blood specimens from a maternal iron dextran (Imferon) treated group and a control group

	Mothers		Babies	
	Treated group (15)	Control group (19)	Treated group (15)	Control group (19)
Hb (g/dl)	12.4 $\pm$ 0.98	12.8 $\pm$ 1.15†	17.2 $\pm$ 1.29	16.4 $\pm$ 1.64
Transferrin (g/l)	3.17 $\pm$ 0.72	3.86 $\pm$ 0.72	2.09 $\pm$ 0.34	2.01 $\pm$ 0.34
Serum iron (μ mol/l)	20.9 $\pm$ 6.59	23.5 $\pm$ 7.76	37.0 $\pm$ 8.5	34.4 $\pm$ 8.74
Transferrin saturation (%)	29.3 $\pm$ 6.69	26.3 $\pm$ 9.67	80.8 $\pm$ 22	79.4 $\pm$ 24.4

Conversion: SI to traditional units—Serum iron $1 \mu\text{mol/l} \approx 5.59 \mu\text{g}/100 \text{ ml}$.
† $p < 0.001$.

Table 2 Results (means $\pm$ SD) of bacteriological tests on babies exposed to maternal iron dextran (Imferon) and control groups

	Iron dextran treated group (13)	Control group (15)
Serum:		
Opsonisation—bactericidal method 2.5% serum (% organisms killed)	84.4 $\pm$ 10.4	94.8 $\pm$ 8.24*
10% serum (% organisms killed)	97.9 $\pm$ 1.79	99.3 $\pm$ 0.39†
Bacteriostatic effects 1/2 dilution (bacterial multiplication at $5 \frac{1}{2}$ h) ($\times 10^4/\text{ml}$)	11.8 $\pm$ 3.02	5.2 $\pm$ 2.19‡
Blood phagocytes:		
Bactericidal capacity (% organisms killed)	97.8 $\pm$ 0.19	99.8 $\pm$ 0.07
Nitroblue tetrazolium stimulated by glass adherence (% positive cells)	73.55 $\pm$ 9.4	72.84 $\pm$ 5.61
Nitroblue tetrazolium stimulated by zymosan (% positive cells)	97.2 $\pm$ 7.26	96.9 $\pm$ 4.55

* $p < 0.005$.

† $p < 0.01$.

‡ $p < 0.001$.

contain bacterial multiplication than controls ($p < 0.001$). In addition the opsonising bactericidal functions at 2.5% and 10% are lower in the test group ($p < 0.005$ and < 0.01 respectively). No pathogenic organisms were grown from the stools of any baby tested.

No significant correlation was shown between the measured iron status of the babies, including saturation of transferrin and their humoral responses. The time from TDI to delivery (range 23-148 days) did not effect serum iron or transferrin concentrations of the mother or baby, which conflicts somewhat, with the finding of Lappin *et al.*,⁹ that the serum iron increased by a factor of six one week after TDI and remained 50% above the basal level at four weeks. Nor was there a relation between the dose of iron dextran to the mother, or the time of infusion before delivery and the babies' humoral responses. No baby, in either group, developed clinical infection in the immediate post-natal period.

Iron is known to be essential for bacterial growth and is an important factor in bacterial pathogenicity.¹⁰ Much information has also been acquired concerning the role of the iron-binding proteins, transferrin and lactoferrin, in providing a defence against bacterial infection.¹¹ Also, saturation of the serum transferrin abolishes both the bactericidal and bacteriostatic effects of normal serum against *E. coli*.¹² Treatment with iron dextran would be expected to saturate transferrin-binding sites. However, this may not occur,¹³ even at the high serum levels achieved during treatment with intravenous iron dextran. Despite these and many other findings, the exact significance of iron and transferrin in the susceptibility of the host to infection, still has to be resolved.^{14 15}

The serum of babies whose mothers received Imferon infusions showed reduced ability to contain bacterial multiplication and also had impaired opsonising function, whereas the bactericidal capacity of blood phagocytes and nitroblue tetrazolium tests were normal. Our results, however, do not agree with the suggestion of Becroft and his colleagues¹ that a high serum iron concentration after intramuscular injection of iron dextran in neonates causes depression of their humoral responses, as we found no significant difference in serum iron concentrations between treated and untreated control groups. There was however a significant reduction in the concentration of transferrin in our treated mothers possibly resulting from suppressed synthesis following iron overload.¹⁶ The babies transferrin concentrations were similar in both groups studied. It is important to remember that strict comparison of results obtained by Becroft and his colleagues¹ and our study is difficult. We

studied the indirect effect of iron on babies whose mothers had received iron dextran while Becroft examined the direct effect on neonates themselves injected with Imferon. This may account for the differences in results.

The importance of our findings in this small group is difficult to assess. Similar abnormalities in neonatal humoral response after iron dextran injections given to babies appear to be associated with an increased risk of *E. coli* meningitis.^{1 2} However, none of our infants developed clinically important infections in the immediate neonatal period. Whether the humoral differences were transient or long-lasting was not determined and further study with prolonged follow-up is needed. It would also be important to test the mothers' immunological responses. Further, the effect on a preterm baby, born soon after TDI, may be even more marked.

Conclusion

The administration of iron dextran complex by TDI to pregnant mothers, has no effect on the serum iron or transferrin concentrations of their babies. It does however, significantly depress the babies' ability to cope with *E. coli* infection in vitro and may put them at risk, at least in the immediate neonatal period.

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