Intramuscular iron-dextran and susceptibility of neonates to bacterial infections

In vitro studies

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SUMMARY An increased incidence of *E. coli* sepsis has been observed in neonates given intramuscular iron-dextran for prevention of iron deficiency. Mechanisms for this apparent effect on susceptibility to infection were investigated by comparing phagocytic and antibacterial functions in paired samples of venous blood from 7 infants, median age 5 days, before and after iron-dextran. Post-treatment sera had increased inhibitory effects on leucocyte chemotaxis and markedly reduced bacteriostatic effects against *E. coli*. The clinical relevance of the effects on chemotaxis is uncertain. The reduction in serum bacteriostasis is similar to that observed in other forms of hyperferraemia not associated with saturation of transferrin, and is a likely cause of the increased susceptibility to infection *in vivo*. We consider that prophylactic treatment with parenteral iron-dextran is contraindicated in early infancy.

The prevention of iron deficiency in 'high risk' infant populations by giving iron-dextran intramuscularly to babies in maternity hospitals was advocated in New Zealand after favourable results were reported from pilot studies (Tonkin, 1970; Cantwell, 1972). However, Barry and Reeve (1973) reported a major increase in the incidence of *E. coli* meningitis and septicaemia in Polynesian neonates receiving prophylactic treatment and a fall in incidence when the practice was stopped (Barry and Reeve, 1976). In Auckland, at the National Women's Hospital, two injections of 1 ml iron-dextran (each containing 50 mg iron) were given during the first week of life to Polynesian infants and others considered at risk (Farmer, 1973). 21 cases of *E. coli* meningitis occurred in 1971 and 1972, compared with one or two per annum previously and in 18 affected infants the onset of symptoms was within 2 to 5 days of the injection of iron-dextran (Farmer and Becroft, 1976). After 1972 a progressive decrease in the use of iron-dextran has coincided with a decline in *E. coli* sepsis.

Because of this circumstantial evidence that prophylactic treatment with iron-dextran was causing increased susceptibility to bacterial infections, we performed *in vitro* studies of a number of factors concerned with 'first-line' resistance to bacterial infection on infants before and after this treatment.

Materials and methods

Seven infants receiving iron-dextran (Imferon) 1 ml intramuscularly on 2 consecutive days were studied. Birthweights ranged from 2.62 kg to 4.46 kg (median 3.20 kg) and treatment began when they were aged between 1 and 54 days (median 5 days). Paired specimens of venous blood were obtained 1 hour before the first injection and 24 hours after the second injection. Control blood samples were obtained from healthy adult volunteers. Leucocyte-rich plasma was obtained from heparinized blood after dextran sedimentation and the cells were washed twice with Hanks's balanced salt solution. For the study of opsonization by the method of Stossel (1973) the cells were also washed in 0.87% NH_4Cl . The concentrated phagocytes (neutrophils and monocytes) were counted and resuspended in buffer solutions at dilutions appropriate for each test. Serum was separated from clotted blood and stored at -170°C . Drops of fresh blood were allowed to clot on glass slides for the nitroblue-tetrazolium tests. The following functions were compared in pre- and post-treatment specimens, the cells of necessity on different days, but paired sera always in parallel.

Cellular.

Bactericidal capacity of venous blood phagocytes

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against *E. coli* (strain 026/B6) was studied by the method of Quie *et al.* (1967).

Chemotactic responsiveness of venous blood phagocytes was studied in a modified Boyden chamber (Markit Engineering, Chicago) by the methods described by Soriano *et al.* (1973).

Nitroblue-tetrazolium (NBT) tests were performed on capillary blood phagocytes adherent to glass by methods described previously (Becroft *et al.*, 1975).

Humoral.

Opsonization. The opsonizing properties of sera were studied: (a) by modification of the bactericidal test of Quie *et al.* (1967) to compare the effect of different sera, each at 10% and 2.5% final concentrations, on the killing of *E. coli* O26/B6 by normal washed neutrophils; and (b) by the method of Stossel (1973) which compares the effect of different sera on the phagocytosis by normal neutrophils of paraffin oil particles coated with *E. coli* O26/B6 lipopolysaccharide (Difco).

Chemotaxis. Serum factors in chemotaxis were studied in the modified Boyden chamber as above, but using normal neutrophils and the serum under study either to generate the chemotactic stimulus, or to suspend the neutrophils and so test for inhibitory factors.

Bacteriostatic effects of serum. The ability of different sera to support the growth of *E. coli* O26/B6 was compared in an incubation mixture of Hanks's solution containing 0.1% gelatin, a standard number of organisms (approximately 10^8 /ml) and each serum, previously heated at 56°C for one hour, at final dilutions of 1:2, 1:20, and 1:100. Each incubation mixture was sampled at intervals during a 5½ hour incubation, the bacteria were counted by a plating technique, and the numbers compared with the count at the beginning of the incubation to give a multiplication factor.

Other studies. Serum iron was measured by an automated method. Serum transferrin and immunoglobulins were measured by radial immunodiffusion (Behringwerke).

Results

Immunoglobulin and transferrin levels were within the normal ranges for age and did not change with treatment. The results of other studies are presented in the Table and Fig. Small sample sizes limited the number of studies performed on some patients. Very high levels of iron were recorded in post-treatment

Fig. Bacteriostatic properties of inactivated sera at 1:2 dilution. Mean of results from 7 infants showing the growth of *E. coli* O26/B6 in serum-free medium, the bacteriostatic effect of adult control sera, the lesser effect of the infants' sera before treatment with iron-dextran, and the further loss of bacteriostasis in post-treatment sera.

sera. The bactericidal capacity of neutrophils against *E. coli* and NBT reduction by stimulated neutrophils did not change with treatment. As expected from the findings of Miller (1971), the neutrophils of these young infants responded less well to a chemotactic stimulus than those of adults. The further decrease in responsiveness post-treatment was inconstant and statistically nonsignificant. Pre- and post-treatment sera had similar opsonizing capacity in the two test systems used. However, all infant sera had lesser opsonizing capacity than adult sera when tested at 2.5% dilution by the bactericidal method (cf McCracken and Eichenwald, 1971), whereas no difference was found using the method of Stossel (1973), which is claimed to assess the alternate pathway of complement activation.

There was no significant difference between the ability of pre- and post-treatment sera to generate a chemotactic stimulus for normal neutrophils which, in contrast to the results obtained by Miller (1971), was greater than that obtained with adult sera. There was a significant ($P < 0.05$) inhibitory effect of post-treatment sera on the ability of normal neutrophils to respond to a chemotactic stimulus. The bacteriostatic effects of inactivated pretreatment sera on *E. coli* were, on average, slightly less than those of control sera. Post-treatment sera at all three dilutions tested showed further significant ($P < 0.001$) reductions in bacteriostatic properties. The results at 1:2 dilution are presented in the Table and Fig.

Table Results of paired tests on blood obtained from 7 infants before and after treatment with intramuscular iron-dextran

	Patients			Controls	
	No. of pairs	Pretreatment median (range)	Post-treatment median (range)	n	Median (range)
Serum iron ($\mu\text{g}/100\text{ ml}$)	7	120 (50–200)	3840 (1096–8680)		
Blood phagocytes					
Bactericidal capacity (% organisms killed)	2	99.8 (99.8–99.9)	99.1 (98.8–99.4)	21	99.8 (98.0–100)
Chemotactic response (cells/high power field)	*	9 (4–32)	5 (2–10)	18	43 (35–55)
NBT stimulated by glass adherence (% positive cells)	7	73 (50–80)	77 (54–81)	10†	44 (34–82)
NBT stimulated by zymosan (% positive cells)	7	98 (80–100)	98 (92–100)	10†	92 (84–99)
Serum					
Opsonization-bactericidal method 2.5% serum (% organisms killed)	3	94 (68–98.3)	86 (84–92)	18	99.4 (80–99.9)
10% serum (% organisms killed)	3	99.3 (98.8–99.4)	99.6 (98.7–99.8)	18	99.8 (98–100)
Opsonization—method of Stossel, (1973) (optical density)	7	0.07 (0.02–0.12)	0.07 (0.01–0.13)	14	0.08 (0.03–0.14)
Generation of chemotactic stimulus (cells/high power field)	7	72 (34–81)	66 (27–84)	18	43 (35–55)
Presence of chemotactic inhibitors (cells/high power field)	7	36 (24–43)	26 (13–43)	18	43 (35–55)
Bacteriostatic effects: 1:2 dilution—(bacterial multiplication at 5½ h)	7	2.4 (0.7–4.1)	12.6 (2.1–66.1)	6	1.4 (1.2–2.6)

*2 paired, 4 unpaired pretreatment, and 2 unpaired post-treatment samples.

†Not concurrent.

Conversion: Traditional units to SI—Serum iron: $1\text{ }\mu\text{g}/100\text{ ml} \approx 0.179\text{ }\mu\text{mol/l}$.

Discussion

Iron-dextran given parenterally could alter susceptibility to bacterial infection either because of its iron content, or because of the macromolecular nature of the complex, and the effects could be either cellular or humoral. Süveges and Glávits (1975) showed an increased susceptibility to *E. coli* infection in mice treated with iron-dextran parenterally, an effect which they considered might be due to reticulo-endothelial blockade by the macromolecule. However, many iron-containing compounds of small molecular size given parenterally also increase the susceptibility of experimental animals to a variety of bacteria (Bullen and Rogers, 1969; Polk and Miles, 1971). Quie (1972) suggested that iron compounds can reduce intracellular bacterial killing and impair intracellular oxidative mechanisms in phagocytes. Conversely, Weinberg (1974), after reviewing the extensive literature on experimental hyperferræmia and increased susceptibility to infection, stated that the association is not due to effects on mobilization or activity of phagocytes, on antibody production, complement activity, or on the toxicity of dead organisms. Our studies on infants treated with iron-dextran support Weinberg's opinion in part, in that there was no impairment of the bactericidal properties of their circulating phagocytes

against *E. coli*, or change in the cell's capacity to reduce NBT. Post-treatment sera inhibited the cellular response to a chemotactic stimulus to a statistically significant degree, but the relevance of this change *in vivo* is uncertain.

Our other positive finding, that treatment with iron-dextran caused a highly significant reduction in the bacteriostatic effects of inactivated serum against *E. coli*, may have greater relevance to the clinical problem. Recent reviews have stressed the importance of iron as an essential bacterial metabolite and the ability to acquire iron as a factor in bacterial pathogenicity (Rogers, 1973; *Lancet*, 1974; Weinberg, 1974). The ability of ionizing iron compounds to reduce the normal antibacterial properties of serum has been attributed to the saturation of transferrin, the major iron chelator in serum (Schade and Caroline, 1946). A similar mechanism has been proposed for the effect of many iron compounds in increasing bacterial virulence in experimental animals and for an increased incidence of bacterial infections in hyperferræmic states in man (*Lancet*, 1974; Weinberg, 1974). However, administration of iron-dextran may not saturate transferrin, even at the high serum-iron levels recorded in our patients (Cox *et al.*, 1968; Henderson and Hillman, 1969). Therefore the effects of iron-dextran are more likely to be analogous to that of poorly ionized iron compounds,

such as haem, which do not saturate transferrin but nevertheless increase the susceptibility of animals to Gram-negative infections (Bullen *et al.*, 1968).

Fletcher (1971) found that the addition of haemoglobin stimulated growth of *E. coli* in heat-inactivated human serum and attributed this effect to the provision of iron as an essential metabolite. In contrast, haemoglobin did not interfere with the complement-dependent bactericidal properties of fresh serum, an effect requiring addition of free iron in excess of the binding capacity of transferrin. In further studies we have shown that the addition of iron-dextran to human serum *in vitro* to give iron levels of 2–4 mg/100 ml (0.358–0.716 mmol/l) reverses bacteriostasis for *E. coli* while bactericidal properties are unaltered, and we have shown a similar selective loss of bacteriostasis in the serum of children who have been treated with iron-dextran for iron deficiency.

Our results suggest that the administration of large amounts of iron-dextran to neonates provides sufficient circulating iron to fill the nutritional requirements of *E. coli* and thus could cause a significant increase in systemic infections in a population having a known susceptibility to these organisms. We believe that there is sufficient evidence from epidemiological and laboratory studies to indicate dangers in such prophylactic regimens for the prevention of iron deficiency.

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