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The effect of parenteral iron administration on the development of *Staphylococcus aureus*-induced experimental pyelonephritis in rats

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Summary. The first of the three groups of rats was taken as a control and the other two groups were injected with high (15 mg/kg) and low (5 mg/kg) doses of ferric ammonium citrate given intramuscularly twice daily for 5 days. Pyelonephritis was produced in all groups by intravenous inoculation with *Staphylococcus aureus*. Serum and urine of each rat was collected periodically and their iron content was determined. The severity of pyelonephritis was evaluated by determination of bacterial growth and pathological lesions in kidneys after 10 days of bacterial inoculation. The results showed that parenteral iron administration markedly aggravated pyelonephritis development in rats. But there was no significant difference in the severity of pyelonephritis between rats treated with high or low iron doses.

Keywords: *Staphylococcus aureus*, experimental pyelonephritis, iron, aggravation of infection

The virulence-enhancing and pathogenicity-promoting effect of iron is well known in various bacteria and neoplasia (Bullen 1981). The effect of iron on virulence and pathogenicity of Gram-negative enteric pathogens has been extensively studied and the mechanisms of acquisition of iron from the environment have been elucidated (Weinberg 1984; Payne 1988). However, few studies concerning the interactions between iron and Gram-positive *Staphylococcus* have been reported and some contradictory results have been obtained. For example it has been observed in in-vitro tests that *Staphylococcus epidermidis* required iron for growing in human serum (Schade 1963) and yet the addition of transferrin markedly inhibited it (McFarlane *et al.* 1970), but *Staphylococcus aureus* grew well in serum without iron addition and transferrin did not affect it. Moreover, parenteral injection of iron results in growth of *Staphylococcus albus* with the development of frank renal abscesses in rats (Fletcher & Goldstein 1970). In our previous studies we observed that a well characterized pyelonephritis can be produced in rats by intravenous inoculation of *Staphlococcus aureus* (Öbek *et al.* 1973; Güngör *et al*, 1981). The present study was designed to investigate the effect of parenter-

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ally injected iron on the development of pyelonephritis induced by *Staphylococcus aureus* and whether there was any correlation between serum and urine iron levels with the severity of infection.

Materials and methods

Animals

Adult male Wistar rats 200–225 g in weight were used in this study. Animals were kept in individual cages and allowed free access to food and tap water throughout the study.

Microorganisms

The *Staphylococcus aureus* strain used in this study was obtained from the Center for Research and Application of Culture Collections of Microorganisms (KUKEM) of Istanbul Medical Faculty. It was grown overnight in trypticase soy broth and suspended in physiological saline solution to the appropriate concentrations.

Iron

A stock solution of ferric ammonium citrate at concentration of 2·8 g/100 ml was prepared by dissolving in sterile saline and stored at 4°C. Iron solutions at desired concentrations were prepared daily by further dilution.

Experimental design

Thirty rats were randomly divided into three groups of 10 rats each. The first group was taken as control and injected intramuscularly with physiological saline in a volume of 0·1 ml/100 g body weight for 5 days. The other two groups were injected with two different doses of ferric ammonium citrate, either 5 or 15 mg/kg, intramuscularly twice daily for 5 days. Pyelonephritis was produced in rats of all groups by inoculation of 1×10^6 *Staphylococcus aureus* intravenously 24 h after the first iron injection.

Blood and urine were collected from all rats of all groups at intervals following iron treatment and the iron levels in serum and urine samples were determined using atomic absorption spectroscopy. On the 10th day the animals were sacrificed and their kidneys were examined macroscopically for abscesses. One kidney from each rat was removed aseptically, weighed, and homogenized in a glass homogenizer. Serial tenfold dilutions of homogenized kidney tissues were made on nutrient agar plates and the bacterial count per gram of wet tissue was determined for each rat. The bacterial count was expressed as $\log_{10}$ colony forming units (CFU) per gram of wet kidney tissue. The remaining kidneys were immersed in 10% neutral formalin, processed for paraffin embedding, sections cut on a microtome, and stained with haematoxylin and eosin. The macroscopic and microscopic examinations of kidneys were done by an experienced pathologist and the pathological findings were rated as follows: no lesion, 0; only microscopic lesions, 1; few lesions seen macroscopically, 2; multiple abscess formation, 3; and widespread abscess formation, 4.

Statistical analysis

The mean values and their standard deviations of serum and urine iron levels, bacterial counts as $\log_{10}$ CFU per gram of kidney tissue, and pathological scores of each group, were calculated. All data are shown as means ± standard deviation of means. Student's *t*-test was used to evaluate significant differences between groups. A *P*-value of $\leqslant 0{\cdot}05$ was considered to be significant.

Results

Serum and urine iron levels were determined at various time intervals in control rats and in rats which were injected intramuscularly with two different doses (5 or 15 mg/kg) of ferric ammonium citrate daily for 5 days. Figure 1 demonstrates that the serum and urine iron levels of control rats did not

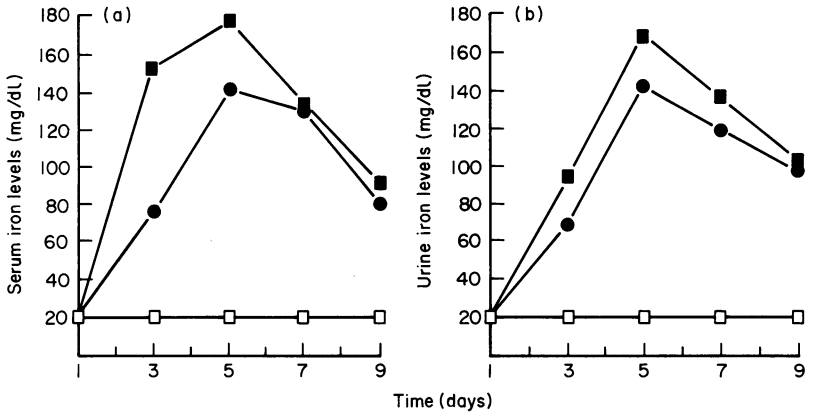

Fig. 1. a. Mean serum and b, mean urine iron levels of □, control rats and rats treated with ●, low (5 mg/kg) and ■, high (15 mg/kg) doses of ferric ammonium citrate injected daily for 5 days.

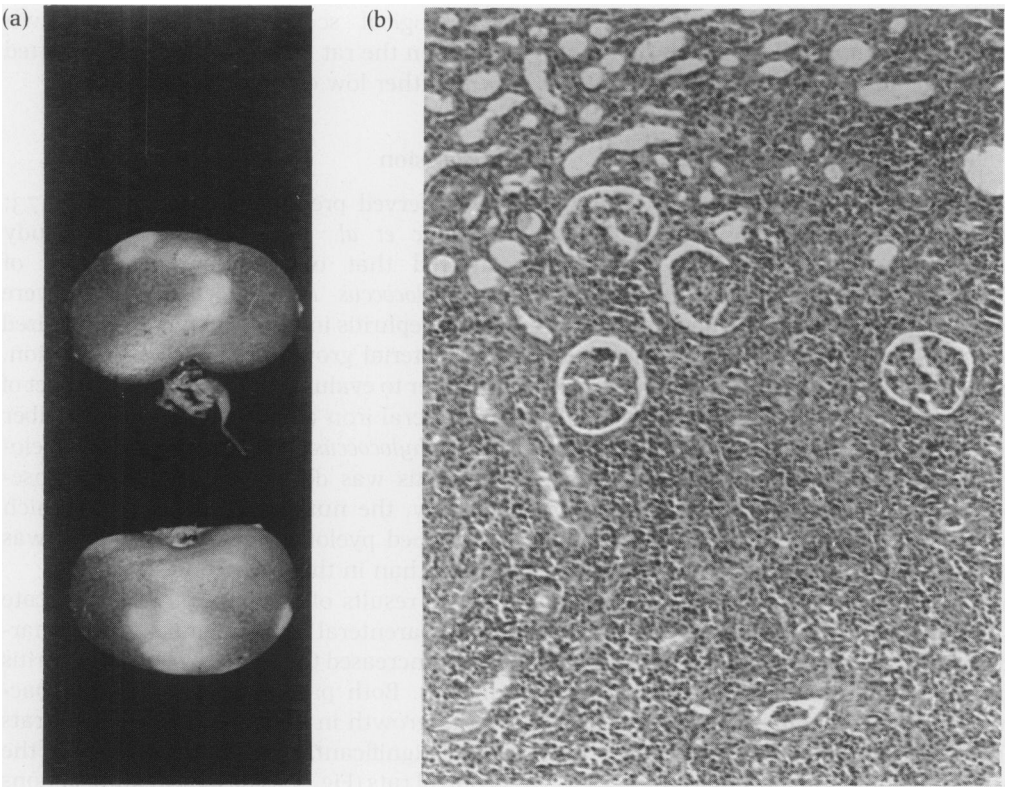

Fig. 2. a, Macroscopic and b, light microscopic section of kidney from rat with pyelonephritis which was induced by intravenous inoculation of 1×10^6 *Staphylococcus aureus.*

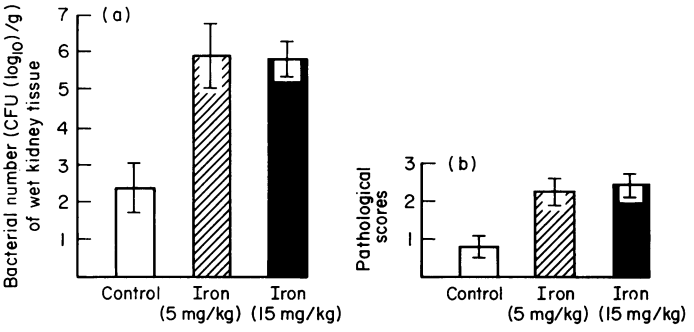

Fig. 3. a, Mean bacterial numbers and b, pathological scores of control and iron-treated rats. Iron was injected as ferric ammonium citrate daily for 5 days. Determinations of viable bacterial number and pathological scores in kidneys were described in text.

change significantly during the study. But in iron-treated rats both serum and urine iron levels promptly increased doses dependently and reached their peak levels at the fifth day. The serum and urine iron levels in iron-treated rats were significantly higher than those of the controls throughout the study. Moreover the serum and urine iron levels of rats treated with high iron doses (15 mg/kg) were also higher than those receiving low doses (5 mg/kg).

The intravenous inoculation of *Staphylococcus aureus* resulted in the development of severe pyelonephritis in the rats. The pyelonephritis was characterized by pyogenic abscess formation and bacterial growth, usually limited to kidneys. Abscesses of different sizes and shapes extended from cortex to renal medulla (Fig. 2a). Histological examination revealed that each abscess consisted of tissue fibrosis and scarring with cell necrosis in the centre surrounded by severe inflammatory cell infiltration (Fig. 2b). Dilated tubules and vessels contained mono and polymorphonuclear leucocytes, lymphocytes and amorphous eosinophilic material. Cellular infiltration and tissue alterations were also observed in some interstitial areas.

Both pathological scores and bacterial growth (Fig. 3) in kidneys of iron-treated rats were found to be significantly higher than those of untreated control rats. There was no significant difference, however, in either the pathological scores or bacterial growth between the rat groups which were injected with either low or high iron doses.

Discussion

As observed previously (Öbek *et al.* 1973; Güngör *et al.* 1981), the present study showed that intravenous inoculation of *Staphylococcus aureus* produced a severe pyelonephritis in rats which is characterized by bacterial growth and abscess formation. In order to evaluate any aggravating effect of parenteral iron administration, the number of *Staphylococcus aureus* used to induce pyelonephritis was deliberately reduced. Consequently, the number of control rats which developed pyelonephritis in this study was lower than in the previous studies.

The results of the present study indicate that parenteral iron administration markedly increased the severity of pyelonephritis in rats. Both pathological scores and bacterial growth in kidneys of iron-treated rats were significantly higher than those of the control rats (Fig. 3). The aggravating actions of iron in urinary tract infection has been observed in some previous studies as well. It has been reported that a single injection of

iron causes an increase in urinary cell excretion in patients with chronic pyelonephritis (Briggs *et al.* 1963). Enhanced resistance to pyelonephritis induced by *Proteus mirabilis* was observed in rats fed on iron-deficient diet (Hart *et al.* 1982). Administration of iron led to formation of renal abscesses in rats inoculated with *Escherichia coli*, *Mycobacterium fortuitum* and *Staphylococcus albus* (Fletcher & Goldstein 1970).

Both serum and urine iron levels progressively increased dose dependently in iron-injected rats. But there was no significant difference in severity of pyelonephritis between treated rats with high or low iron levels in their serum and urine.

Thus our results suggest that increased tissue iron levels could induce marked aggravation of pyelonephritis, but there may be no close correlation between tissue iron levels and severity of infection.

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