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# Effects of Ascorbic Acid on Interactions between Ciprofloxacin and Ferrous Sulphate, Sodium Ferrous Citrate or Ferric Pyrophosphate, in Mice

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**Abstract**

The absorption of ciprofloxacin has been reported to be impaired by concomitant administration of ferrous sulphate. The effects of sodium ferrous citrate and ferric pyrophosphate, which have been used as extensively as ferrous sulphate, on the absorption of ciprofloxacin were compared with that of ferrous sulphate. The effects of ascorbic acid on the interactions between ciprofloxacin and each iron compound were studied in mice. Mice were treated orally with ciprofloxacin ($50\,mg\,kg^{-1}$) alone, the iron compound (ferrous sulphate, sodium ferrous citrate or ferric pyrophosphate; 50 mg elemental iron $kg^{-1}$) alone, ciprofloxacin with each iron compound or ciprofloxacin in combination with each iron compound and ascorbic acid ($250\,mg\,kg^{-1}$).

The maximum serum concentration of ciprofloxacin was significantly ($P < 0{\cdot}01$) reduced from $1{\cdot}15 \pm 0{\cdot}11\,\mu g\,mL^{-1}$ (ciprofloxacin alone) to $0{\cdot}17 \pm 0{\cdot}01$, $0{\cdot}27 \pm 0{\cdot}01$ or $0{\cdot}28 \pm 0{\cdot}02\,\mu g\,mL^{-1}$, respectively, when ferrous sulphate, sodium ferrous citrate or ferric pyrophosphate was administered along with ciprofloxacin. The addition of ascorbic acid did not affect the inhibitory effects of each iron compound on the absorption of ciprofloxacin. Ciprofloxacin did not affect the variation of serum iron levels after administration of each iron compound. The addition of ascorbic acid significantly ($P < 0{\cdot}01$) enhanced the increase in serum iron concentration after administration of sodium ferrous citrate, showing an increase from $270 \pm 6\,\mu g\,dL^{-1}$ to $463 \pm 11\,\mu g\,dL^{-1}$ compared with an increase from $248 \pm 8\,\mu g\,dL^{-1}$ to $394 \pm 18\,\mu g\,dL^{-1}$ after administration of sodium ferrous citrate alone. Ascorbic acid also caused a significant ($P < 0{\cdot}01$) increase in serum iron concentration from $261 \pm 16\,\mu g\,dL^{-1}$ to $360 \pm 12\,\mu g\,dL^{-1}$ after administration of ferric pyrophosphate, although it did not affect the levels after ferrous sulphate administration.

The results suggest that sodium ferrous citrate and ferric pyrophosphate should not be administered with ciprofloxacin (as for ferrous sulphate) and that sodium ferrous citrate is converted to the ferric form more easily than ferrous sulphate. This difference in convertibility might contribute to a clinical difference between sodium ferrous citrate and ferrous sulphate.

Ciprofloxacin is a broad spectrum antibacterial agent which shows excellent activity against a wide range of Gram-positive and Gram-negative bacteria. Previous studies have shown that the absorption of ciprofloxacin is impaired by the concomitant administration of drugs containing iron, such as ferrous sulphate and ferrous gluconate (Kara et al 1991; Lehto et al 1994). Sodium ferrous citrate and ferric pyrophosphate are other iron compounds that have been used as extensively as ferrous sulphate. The chemical characteristics of these drugs are different from that of ferrous sulphate, as sodium ferrous citrate is a non-ionized iron compound (Fujita 1982) and ferric pyrophosphate is a ferric compound. The effects of these iron compounds on the absorption of ciprofloxacin are unknown.

Regarding the mechanism of interaction between ciprofloxacin and iron compounds, the formation of

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a ferric iron–ciprofloxacin complex is thought to be the cause of the reduction in ciprofloxacin bioavailability in the presence of iron (Kara et al 1991). Therefore, the effects of non-ionized iron compounds and ferric compounds on the absorption of ciprofloxacin might differ from that of the ferrous compounds, ferrous sulphate and ferrous gluconate. Furthermore, ascorbic acid, which causes reduction of iron from the ferric to the ferrous form, may also influence the interactions between ciprofloxacin and each iron compound.

In this study, the effects of sodium ferrous citrate and ferric pyrophosphate on the absorption of ciprofloxacin were compared with that of ferrous sulphate, and the effects of ascorbic acid on the interactions between ciprofloxacin and each iron compound were studied in mice.

## Materials and Methods

*Drugs*

The drugs used were ciprofloxacin (Bayer Yakuhin, Osaka, Japan), ferrous sulphate (Nacalai Tesque, Kyoto, Japan), sodium ferrous citrate (Sansho, Saitama, Japan), ferric pyrophosphate (Sigma, St Louis, MO), and ascorbic acid (Nacalai Tesque). All other chemicals were of the highest purity available, and obtained from Nacalai Tesque. Ciprofloxacin, ferrous sulphate, sodium ferrous citrate, ferric pyrophosphate and ascorbic acid were separately dissolved in 0·5% carboxymethylcellulose solution. The final concentrations of the drugs were 6·25 mg $mL^{-1}$ for ciprofloxacin, 6·25 mg elemental iron $mL^{-1}$ for each iron compound, and 31·25 mg $mL^{-1}$ for ascorbic acid.

*Animals*

Male ddY mice, 29–32 g, were obtained from Japan SLC (Shizuoka, Japan) and were kept in an animal room with a 12-h light–dark cycle. They were given free access to a standard pelleted diet and tap water except during the experiments. All experiments were carried out at a temperature of $21 \pm 2°C$.

*Experimental protocol*

Mice were fasted for 12 h before the beginning of the experiment, and were treated orally with ciprofloxacin (50 mg $kg^{-1}$) alone, the iron compound (ferrous sulphate, sodium ferrous citrate or ferric pyrophosphate; 50 mg elemental iron $kg^{-1}$) alone, ciprofloxacin with each iron compound or ciprofloxacin in combination with each iron compound and ascorbic acid (250 mg $kg^{-1}$). When two or three drugs were administered, the agents were administered separately and consecutively within 1 min.

Mice were divided into 4 test groups plus control, and blood samples were obtained by decapitation before drug administration (control) and at 0·5, 1, 2 and 4 h after. Serum samples were separated by centrifugation (1000 *g*) at 20°C for 20 min and were stored at −20°C until analysis.

*Drug assay*

Serum concentrations of ciprofloxacin were measured by the disc agar diffusion microbiological method using *Escherichia coli* kp as the indicator organism. These measurements were performed by BML Inc. (Tokyo, Japan). Total serum iron concentrations were measured by the direct method based on bathophenanthroline reagent (Fe direct, Shino-Test, Tokyo, Japan). Using this kit, ferrous iron was detected after release of ferric iron from ferritin and transferrin iron in acidic solution followed by conversion to ferrous iron by adding ascorbic acid.

*Statistical analysis*

The data were analysed using Stat View (Abacus Concepts, Berkeley, USA). A two-way analysis of variance was followed by a post hoc Fisher's protected least significant difference test. The level of significance ($P$) was set at 0·01.

## Results

Serum ciprofloxacin concentrations after administration of ciprofloxacin alone, ciprofloxacin with each iron compound, and ciprofloxacin in combination with each iron compound and ascorbic acid are shown in Figure 1. When ciprofloxacin was administered alone, the serum ciprofloxacin concentrations at 0·5 and 1 h after administration were $1·15 \pm 0·11$ $\mu g\ mL^{-1}$ and $0·69 \pm 0·16$ $\mu g\ mL^{-1}$, respectively. When ciprofloxacin was administered with each iron compound, those concentrations were significantly ($P < 0·01$) lower than after administration of ciprofloxacin alone, values being respectively, $0·15 \pm 0·01$ $\mu g\ mL^{-1}$ and $0·17 \pm 0·01$ $\mu g\ mL^{-1}$ in ferrous sulphate treatment; $0·27 \pm 0·01$ $\mu g\ mL^{-1}$ and $0·18 \pm 0·01$ $\mu g\ mL^{-1}$ in sodium ferrous citrate treatment; and $0·28 \pm 0·02$ $\mu g\ mL^{-1}$ and $0·21 \pm 0·01$ $\mu g\ mL^{-1}$ in ferric pyrophosphate treatment. The addition of ascorbic acid did not affect the inhibitory effects of each iron compound on the absorption of ciprofloxacin, giving concentrations of $0·01 \pm$

$0{\cdot}00\,\mu g\,mL^{-1}$ and $0{\cdot}01 \pm 0{\cdot}00\,\mu g\,mL^{-1}$ in the ferrous sulphate group, $0{\cdot}13 \pm 0{\cdot}02\,\mu g\,mL^{-1}$ and $0{\cdot}17 \pm 0{\cdot}03\,\mu g\,mL^{-1}$ in the sodium ferrous citrate group and $0{\cdot}19 \pm 0{\cdot}02\,\mu g\,mL^{-1}$ and $0{\cdot}13 \pm 0{\cdot}02\,\mu g\,mL^{-1}$ in the ferric pyrophosphate group.

Serum iron concentrations after administration of each iron compound alone, each iron compound with ciprofloxacin and each iron compound in combination with ciprofloxacin and ascorbic acid are shown in Figure 2. When ferrous sulphate or

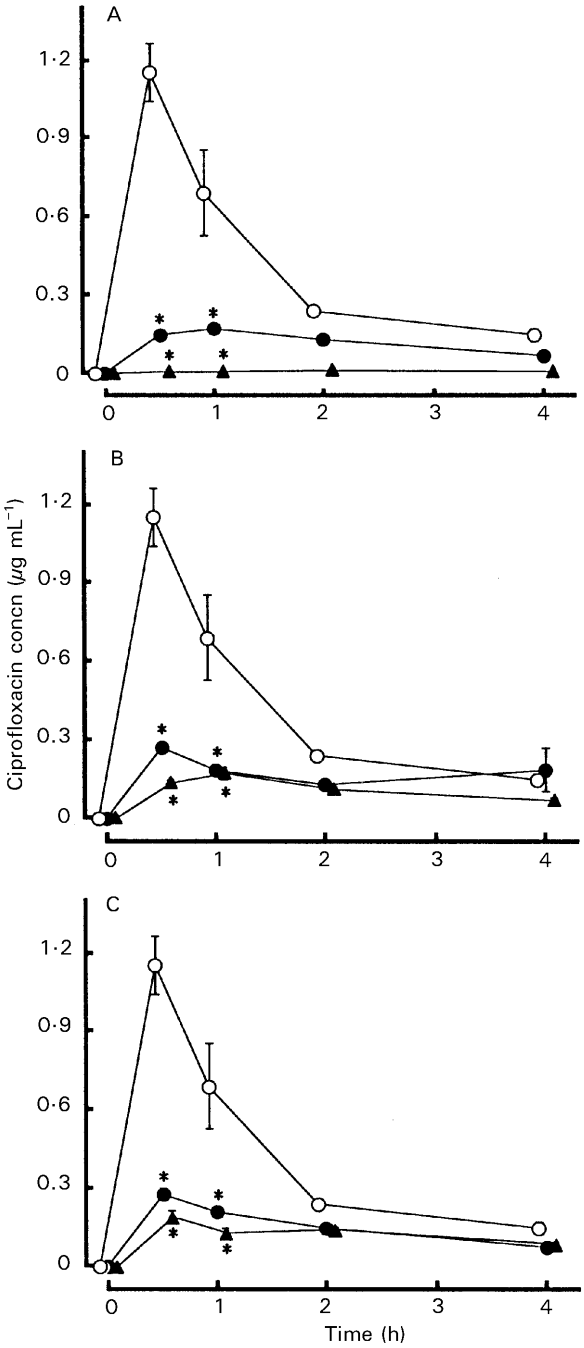


Figure 1. Serum ciprofloxacin concentrations after administration, to mice, of ciprofloxacin alone (○), ciprofloxacin with each iron compound (●): A, ferrous sulphate; B, sodium ferrous citrate; C, ferric pyrophosphate; or ciprofloxacin in combination with iron compound and ascorbic acid (▲). The oral doses of ciprofloxacin, iron compound and ascorbic acid were $50\,mg\,kg^{-1}$, 50 mg elemental iron $kg^{-1}$ and $250\,mg\,kg^{-1}$, respectively. Values are means ± s.e.m., n = 10. * $P < 0{\cdot}01$, compared with ciprofloxacin alone.

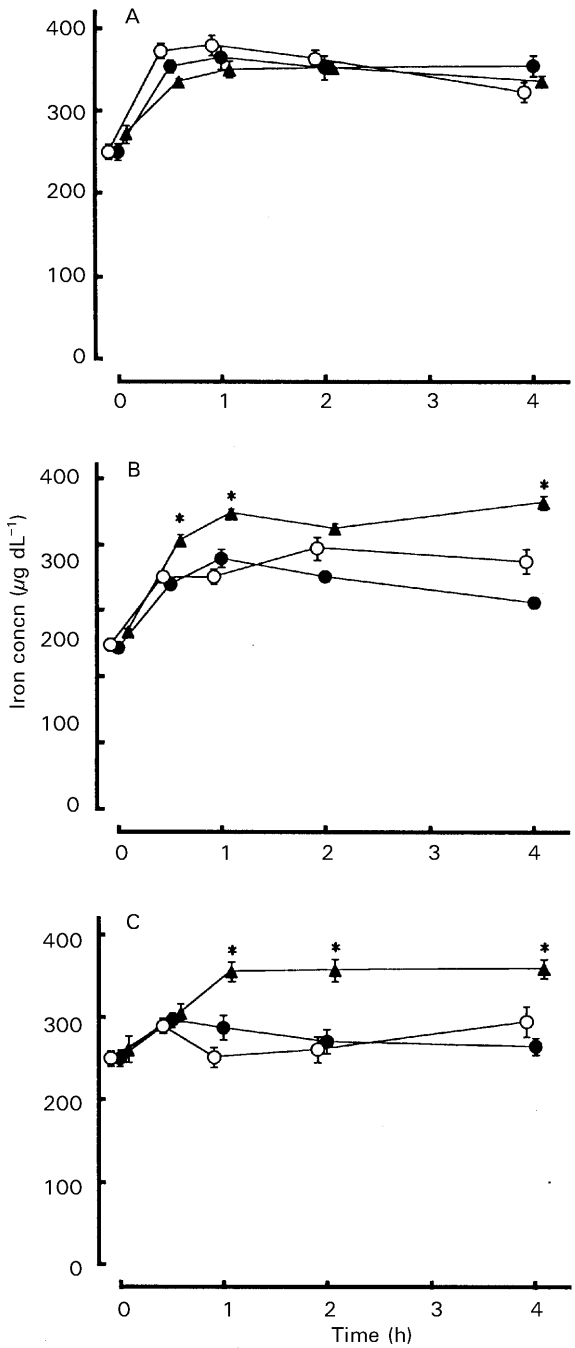


Figure 2. Serum iron concentrations after administration, to mice, of iron compound alone (○): A, ferrous sulphate; B, sodium ferrous citrate; C, ferric pyrophosphate; or iron compound with ciprofloxacin (●), or iron compound in combination with ciprofloxacin and ascorbic acid (▲). The oral doses of iron compound, ciprofloxacin and ascorbic acid were 50 mg elemental iron $kg^{-1}$, $50\,mg\,kg^{-1}$ and $250\,mg\,kg^{-1}$, respectively. Values are means ± s.e.m., n = 10. * $P < 0{\cdot}01$, compared with iron compound alone.

sodium ferrous citrate was administered alone, the serum iron concentration was significantly ($P < 0{\cdot}01$) increased from $251 \pm 9\,\mu g\,dL^{-1}$ to $379 \pm 12\,\mu g\,dL^{-1}$ and from $248 \pm 8\,\mu g\,dL^{-1}$ to $394 \pm 18\,\mu g\,dL^{-1}$ respectively, although the concentration after administration of ferric pyrophosphate alone did not increase. Ciprofloxacin did not affect the serum iron concentrations after administration of each iron compound. The addition of ascorbic acid significantly ($P < 0{\cdot}01$) enhanced the increase in serum iron concentrations after administration of sodium ferrous citrate, showing an increase from $270 \pm 6\,\mu g\,dL^{-1}$ to $463 \pm 11\,\mu g\,dL^{-1}$, and causing a significant ($P < 0{\cdot}01$) increase from $261 \pm 16\,\mu g\,dL^{-1}$ to $360 \pm 12\,\mu g\,dL^{-1}$ after administration of ferric pyrophosphate, although it did not affect the serum iron concentration after administration of ferrous sulphate.

## Discussion

One of the objectives of this study was to evaluate the effects of sodium ferrous citrate and ferric pyrophosphate on the absorption of ciprofloxacin. Both drugs were found to impair the absorption of ciprofloxacin as intensively as ferrous sulphate. Therefore, it is proposed that sodium ferrous citrate and ferric pyrophosphate should not be administered with ciprofloxacin, as is the case for ferrous sulphate.

If only ferric iron reacted with ciprofloxacin, the addition of ascorbic acid would interfere with the inhibitory effect of iron compounds on the absorption of ciprofloxacin, since ascorbic acid reduces ferric iron to ferrous iron. However, the addition of ascorbic acid did not affect the impaired absorption of ciprofloxacin by an iron compound, although it significantly increased the absorption of iron after administration of sodium ferrous citrate or ferric pyrophosphate. These findings suggest that not only ferric iron, but also ferrous iron, binds ciprofloxacin as proposed for cefdinir and iron (Mooney et al 1995).

We recently reported that sodium ferrous citrate significantly impaired the absorption of cefdinir as intensively as ferrous sulphate (Miyashita et al 1999). It also impaired the absorption of ciprofloxacin in this study. The mechanism by which sodium ferrous citrate inhibits the absorption of these drugs is difficult to understand, if indeed the former drug is not ionized in the gastrointestinal tract as supposed by Terato et al (1973). They suggested that low-molecular-weight polymers of sodium ferrous citrate are transferred from the intestinal lumen into the peripheral bloodstream principally in their original forms. However, our results indicate that sodium ferrous citrate interacts with ciprofloxacin and cefdinir as ferrous sulphate does, suggesting that the ferrous ion, in the structure of sodium ferrous citrate, reacts with ciprofloxacin and cefdinir. Sodium ferrous citrate may be ionized under the high acidity of the stomach. Therefore, it is not clear how the drug impairs the absorption of ciprofloxacin and cefdinir. Additional studies are required to clarify the mechanism.

The effect of ciprofloxacin on the absorption of iron after administration of iron compounds has not been reported, although the inhibitory effect of iron compounds on the absorption of ciprofloxacin has been shown as described above. In this study, iron absorption was found not to be affected by ciprofloxacin. In general, only a fraction of iron administered orally can be absorbed through the intestinal membrane. In this study, the molar concentration of administered iron compounds was higher than that of ciprofloxacin to reflect the situation during therapy. Therefore, it is considered that a large amount of unreacted iron is available for absorption after administration of iron compounds with ciprofloxacin.

The variations in serum iron levels after administration of each iron compound with or without ascorbic acid were interesting. Iron deficiency is shown to enhance ferrireductase activity and to increase iron uptake activity (Raja et al 1992). However, the mice used in this study were not iron deficient. Serum iron concentrations significantly increased after administration of ferrous sulphate or sodium ferrous citrate. Contrarily, after administration of ferric pyrophosphate, the concentrations did not increase. Ferric iron is known to be absorbed after reduction to ferrous iron (Raja et al 1992; Han et al 1995). Interestingly, the addition of ascorbic acid caused a significant increase in serum iron concentrations after administration of ferric pyrophosphate. Therefore, it is likely that ferrireductase activity at the intestinal tract in mice is insufficient for the iron to be absorbed.

The increase in serum iron concentrations after administration of sodium ferrous citrate was enhanced by the addition of ascorbic acid, although the increase after administration of ferrous sulphate was not affected. To understand these results, and those of ferric pyrophosphate, we hypothesized the effects of ascorbic acid on the absorption of iron after administration of each iron compound as follows. That is to say, sodium ferrous citrate is converted to the ferric form more easily than ferrous sulphate in the intestinal tract, while a fraction of the former drug remains in its ferrous form or

original form to be absorbed. The enhancement of iron absorption after combined administration of ascorbic acid and sodium ferrous citrate or ferric pyrophosphate is thought to be due to the reducing effect of ascorbic acid, from ferric to ferrous iron. The failure of ascorbic acid to enhance iron absorption after administration with ferrous sulphate seems due to the principal existence of the drug in ferrous form. This hypothesis seems to proficiently explain the findings of this study, although additional studies are required to confirm the theory.

In summary, sodium ferrous citrate and ferric pyrophosphate impaired the absorption of ciprofloxacin as intensively as ferrous sulphate. The addition of ascorbic acid did not affect the inhibitory effect of each iron compound on the absorption of ciprofloxacin. Ciprofloxacin did not affect the variation in serum iron levels after administration of each iron compound. The addition of ascorbic acid enhanced the increase in serum iron levels after administration of sodium ferrous citrate, and caused an increase after administration of ferric pyrophosphate, although it did not affect the increase after administration of ferrous sulphate. These findings suggest that sodium ferrous citrate is converted to the ferric form more easily than ferrous sulphate. This difference in convertibility might contribute to a clinical difference between sodium ferrous citrate and ferrous sulphate.

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