

Evaluation of serum iron levels during FOLFOX4 and FOLFIRI therapies

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Abstract. FOLFOX4 and FOLFIRI are effective regimens for the treatment of advanced colorectal cancer, and their use together with molecular targeting drugs has recently become more common. In the present study, we evaluated the changes in the serum iron levels of patients undergoing FOLFOX4 or FOLFIRI therapy alone or in combination with bevacizumab (BV). The serum iron level was increased 48 h after therapy and was restored to baseline 2 weeks afterwards in colorectal cancer patients who received FOLFOX4 or FOLFIRI alone or in combination with BV. This transient increase in serum iron was observed repeatedly during chemotherapy. The serum iron level was $71.66 \pm 28.96 \mu\text{g/dl}$ (mean \pm standard deviation) before treatment and significantly increased to $186.82 \pm 83.17 \mu\text{g/dl}$ ($p < 0.001$) 48 h after therapy. A transient increase in serum iron levels was also observed when FOLFIRI was administered to a patient after tumor resection. In contrast, no decrease in blood hemoglobin, no increase in liver enzymes and no increase in urinary iron excretion were observed. Based on these results, it can be concluded that an increase in serum iron may be induced by a transient change in iron distribution within the body after FOLFOX4/FOLFIRI therapy with or without BV.

Introduction

FOLFOX4 therapy [folinic acid (FOL), fluorouracil (F) plus oxaliplatin (OX)] (1) and FOLFIRI therapy (FOL, F, plus irinotecan (IRI)] (2) are international standard treatments for advanced colorectal cancer (3). In recent years, it has also

been recommended that molecular targeting drugs such as bevacizumab (BV) (4,5) or cetuximab (Cet) (6,7) be combined with FOLFOX4 or FOLFIRI.

The median patient survival time (MST) was reported to be significantly longer for FOLFOX4 + BV therapy (12.9 months) than for FOLFOX4 alone (10.8 months), confirming that the addition of BV increased the efficacy of the treatment (4). The MST was also reported to be significantly longer for FOLFIRI + Cet (8.9 months) than for FOLFIRI alone (8.0 months) (8). These observations suggest that chemotherapy combined with molecular targeting drugs is more effective for treatment of advanced colorectal cancer. Thus, the addition of such molecular targeting drugs to chemotherapy has been recommended (Saltz LB, et al: Proc ASCO 170: abs. 4028, 2007).

Anemia is one of the most common adverse effects of chemotherapy. However, Follézou *et al* (9) reported an increase in the serum iron level after the administration of various anticancer drugs, including 5-FU, actinomycin D, adriacin and cyclophosphamide. Yet, there have been no reports concerning the effect of FOLFOX4 and FOLFIRI on serum iron levels. Therefore, in the present study, we evaluated the effect of FOLFOX4 and FOLFIRI therapies on changes in serum levels of iron as well as transferrin and ferritin.

Materials and methods

Subjects. Fifty-eight subjects (92 cases) were enrolled in this study. They were admitted to Tobu Chiiki Hospital (Tokyo Metropolitan Health and Medical Treatment Corporation, Tokyo, Japan) and received FOLFOX4 or FOLFIRI therapy alone or in combination with BV between April 2005 and September 2008. Prior to the enrollment, informed consent was obtained from all the subjects. The patient characteristics are presented in Table I.

Measurement of the serum iron level. Serum iron was measured by the hospital laboratory before and 48 h after treatment in the 44 patients receiving FOLFOX4 therapy. The normal range of serum iron was 60-210 $\mu\text{g/dl}$ for men and 50-170 $\mu\text{g/dl}$ for women. The serum iron level was also measured before and after treatment in the 11 patients receiving FOLFOX4 + BV. Furthermore, serum iron levels were compared before and

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Table I. Characteristics of the subjects.

No. of subjects	58
Gender (male/female)	35/23
Mean age (years)	69.4±7.7
Dukes' stage (A/B/C/D)	1/9/28/20
Colon/rectum	37/21
Recurrence	
Liver	23
Lung	9
Peritoneum	7
Lymph node	5
Local recurrence	5
Bone	1
Brain	1
Other	3
Unresectable	4

after the introduction of BV in the 10 patients who received FOLFOX4 + BV after FOLFOX4 alone.

Serum iron was measured before and after treatment in the 31 patients who received FOLFIRI therapy, and in the 6 patients who received FOLFIRI + BV. The serum iron level was also compared before and after the introduction of BV in the 5 patients who received FOLFIRI + BV after FOLFIRI alone.

Measurement of transferrin and ferritin. Transferrin and ferritin levels were measured before and after treatment at SRL, Inc. (Tokyo, Japan) in the 15 and 14 patients who received FOLFOX4 and FOLFIRI therapy, respectively. The normal range of transferrin was 190-300 mg/dl for men and 200-340 mg/dl for women, while the normal range of ferritin was 39.4-340 ng/ml for men and 3.6-114 ng/ml for women.

Measurement of urinary iron. Urinary iron was measured at the hospital laboratory on the day of treatment and on the next day in 5 and 7 patients who received FOLFOX4 and FOLFIRI therapy, respectively.

Statistical analysis. The t-test was used to compare the two groups, and $p < 0.05$ was considered to be significant. Data are expressed as the mean \pm standard deviation (SD).

Results

Changes in serum iron levels during FOLFOX4 therapy. A typical pattern of the changes in the serum iron levels before and after FOLFOX4 therapy is shown in Fig. 1. The serum iron level transiently increased after treatment (48 h) and then returned to baseline within 2 weeks. In the FOLFOX4 group (44 patients and 272 blood samples), the serum iron level was $68.24 \pm 25.20 \mu\text{g/dl}$ before treatment and increased significantly to $143.34 \pm 62.18 \mu\text{g/dl}$ afterwards ($p < 0.001$, Fig. 2), showing an increase of $238.54 \pm 127.17\%$. In the FOLFOX4 + BV group, the serum iron level also increased transiently after treatment (48 h), and then returned to baseline within 2 weeks (data not shown). In the FOLFOX4 + BV group (11 patients and 46 blood

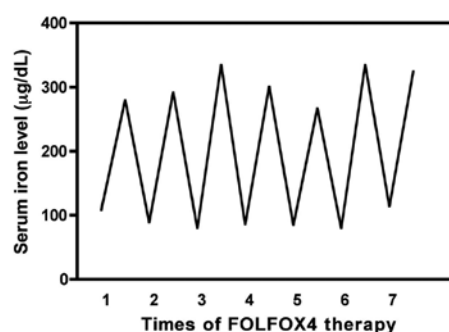


Figure 1. A typical pattern of the changes in the serum iron levels before and after FOLFOX4 therapy.

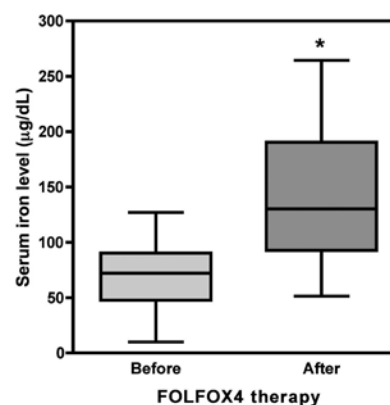


Figure 2. Changes in the serum iron levels before and after FOLFOX4 therapy. Serum iron levels were measured using 272 blood samples from 44 patients. Data are the mean \pm SD, and values are compared before and after FOLFOX4 therapy. * $p < 0.001$.

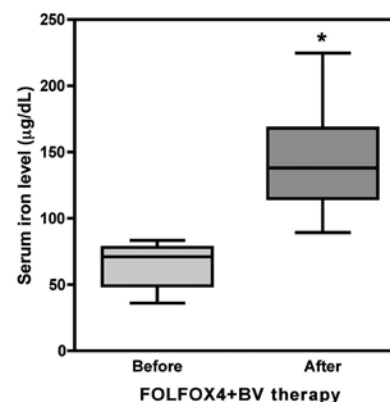


Figure 3. Changes in the serum iron levels before and after FOLFOX4 + BV therapy. Serum iron levels were measured using 46 blood samples from 11 patients. Data are the mean \pm SD, and values are compared before and after FOLFOX4 + BV therapy. * $p < 0.001$.

samples), the serum iron level was $65.59 \pm 15.87 \mu\text{g/dl}$ before treatment and increased significantly to $147.55 \pm 44.55 \mu\text{g/dl}$ after treatment ($p < 0.001$, Fig. 3), showing an increase of $247.16 \pm 60.70\%$.

Changes in serum iron levels during FOLFIRI therapy. A typical pattern of the changes in the serum iron levels before

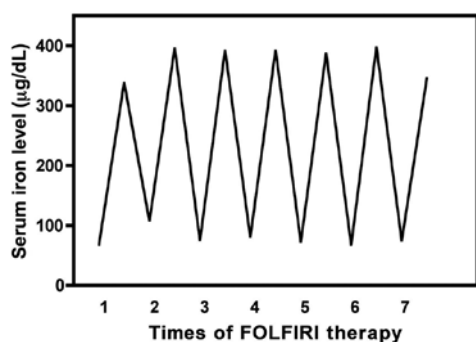


Figure 4. A typical pattern of the changes in the serum iron levels before and after FOLFIRI therapy.

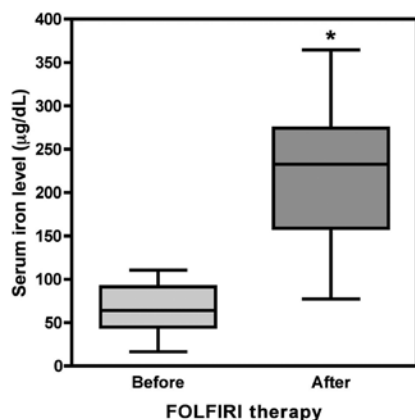


Figure 5. Changes in the serum iron levels before and after FOLFIRI therapy. Serum iron levels were measured using 231 blood samples from 31 patients. Data are the mean \pm SD, and values are compared before and after FOLFIRI therapy. * $p < 0.001$.

and after FOLFIRI therapy is shown in Fig. 4. The serum iron level transiently increased after treatment (48 h) and then returned to baseline within 2 weeks. In the FOLFIRI group (31 patients and 231 blood samples), the serum iron level was 66.01 ± 27.47 $\mu\text{g/dl}$ before treatment and increased significantly to 221.69 ± 78.51 $\mu\text{g/dl}$ afterwards ($p < 0.001$, Fig. 5), showing an increase of $399.94 \pm 6.25\%$. In the FOLFIRI + BV group, the serum iron level also increased transiently after treatment (48 h) and then returned to baseline within 2 weeks (data not shown). In the FOLFIRI + BV group (6 patients and 26 blood samples), the serum iron level was 64.68 ± 23.60 $\mu\text{g/dl}$ before treatment and increased significantly to 244.55 ± 40.54 $\mu\text{g/dl}$ after treatment ($p < 0.001$, Fig. 6), showing an increase of $440.33 \pm 156.22\%$.

Since there was little difference in the changes in serum iron between FOLFOX4 and FOLFIRI therapy when these regimens were combined with BV (Figs. 2, 3, 5 and 6), BV was considered to impart no influence on the changes in iron levels. To confirm this, changes in serum iron were examined in patients who underwent FOLFOX4 + BV after FOLFOX4 alone. No difference was noted in the serum iron levels after treatment between FOLFOX4 alone (80.84 ± 53.94 $\mu\text{g/dl}$, $n = 10$) and FOLFOX4 + BV (76.03 ± 34.84 $\mu\text{g/dl}$). Furthermore, the serum iron levels were also measured in patients who under-

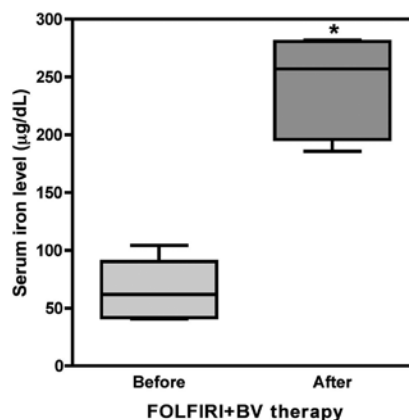


Figure 6. Changes in the serum iron levels before and after FOLFIRI + BV therapy. Serum iron levels were measured using 26 blood samples from 6 patients. Data are the mean \pm SD, and values are compared before and after FOLFIRI therapy. * $p < 0.001$.

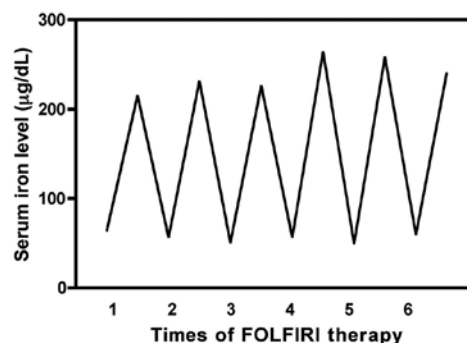


Figure 7. A typical pattern of the changes in the serum iron levels before and after FOLFIRI therapy in a tumor-resected patient.

went FOLFIRI + BV after FOLFIRI alone. Similarly, there was no difference in the serum iron levels after the treatment between FOLFIRI alone (205.09 ± 139.37 $\mu\text{g/dl}$, $n = 5$) and FOLFIRI + BV (257.45 ± 151.63 $\mu\text{g/dl}$).

Changes in transferrin and ferritin levels during FOLFOX4 and FOLFIRI therapies. The influence of chemotherapy on transferrin (an iron-transporting protein) (10) and ferritin (an iron storage protein) (11,12) was also investigated. In the 15 patients of the FOLFOX4 group, transferrin levels were not different before (256.79 ± 80.13 mg/dl) and after (233.53 ± 80.70 mg/dl) the treatment ($p = 0.14$). In the 14 patients of the FOLFOLI group, the transferrin level was 236.15 ± 54.31 mg/dl before the treatment and decreased slightly to 196.50 ± 36.85 mg/dl after the treatment ($p < 0.001$), but these changes were within the normal range of serum transferrin.

In the 15 patients of the FOLFOX4 group, the ferritin levels were not different before (192.32 ± 224.88 ng/dl) and after (210.15 ± 210.16 ng/dl) the treatment ($p = 0.67$). Similarly, in the 14 patients of the FOLFIRI group, the ferritin levels were not different before (211.48 ± 181.83 ng/dl) and after (220.15 ± 182.97 ng/dl) the treatment ($p = 0.83$). These changes were all within the normal range of serum ferritin.

Urinary iron excretion during FOLFOX4 and FOLFIRI therapies. To determine whether the changes in serum iron during chemotherapy were related to the urinary iron excretion, urine samples were collected on the day of treatment and on the next day to measure the urinary iron level in 5 and 7 patients of the FOLFOX4 and FOLFIRI groups, respectively. Although urinary iron excretion was 0.09 mg/day on the day of treatment in 1 subject receiving FOLFIRI, it was within the normal range (<0.2 mg/day). Moreover, urinary iron excretion was below the detection limit (0.03 mg/day) in all of the other subjects.

Discussion

Recently, a powerful and effective combination chemotherapy has become available due to the development of antitumor chemotherapeutic agents and molecular targeting drugs. However, the incidence of serious adverse reactions has also increased. Almost all anticancer agents have the potential to induce myelosuppression by eliciting the apoptosis/necrosis of immature myelopoietic cells. In particular, severe leukopenia, thrombocytopenia and erythropenia are serious adverse events that lead to the termination of treatment. FOLFOX4 and FOLFIRI therapies are standard treatments for advanced colorectal cancer; however, they cause characteristic adverse reactions, such as peripheral neuropathy and severe diarrhea as well as conventional reactions like myelosuppression (13,14).

During our preliminary studies on the adverse events caused by FOLFOX4 or FOLFIRI therapy, an increase in the serum iron level was sometimes observed, while the red blood cell count remained unchanged. Focusing on this finding, the present study was carried out.

In regard to the chemotherapy-induced changes in the serum iron level, Foll  zou *et al* reported that serum iron levels transiently increased during chemotherapy (9). However, their study differed from the present investigation in the following respects. Their patients received relatively older anticancer drugs, such as 5-FU, adriacin, and cyclophosphamide, and they did not measure the levels of transferrin or ferritin. Furthermore, they did not evaluate the effect of tumor cell death and hepatic damage on the increase in serum iron levels.

In the present study, we measured serum iron as well as transferrin and ferritin levels in patients who received FOLFOX4 or FOLFIRI therapy alone or in combination with BV. The serum iron level showed a transient increase in patients receiving FOLFOX4 or FOLFIRI therapy alone. In most of the patients, serum iron increased above the normal range (60-210 $\mu\text{g/dl}$ for men and 50-170 $\mu\text{g/dl}$ for women) and sometimes reached 400 $\mu\text{g/dl}$. We confirmed that the serum iron level similarly increased regardless of the administration of BV, suggesting that the transient increase in serum iron was not due to BV, but was presumably caused by FOLFOX4 or FOLFIRI therapy alone. In contrast, transferrin levels were not essentially changed during chemotherapy with FOLFOX4 or FOLFIRI. Moreover, ferritin levels were not basically changed by FOLFOX4 and FOLFIRI therapies.

Levels of aspartate aminotransferase, alanine aminotransferase and hemoglobin did not change during chemotherapy

(data not shown). In addition, urinary excretion of iron was not increased by the chemotherapy. These observations suggest that the transient increase in serum iron was not due to the destruction of hepatocytes or erythrocytes. However, it is possible that iron was transiently released from tumor cells into the blood by chemotherapy. To examine this possibility, FOLFIRI therapy was administered to a patient after tumor resection (1 patient and 6 blood samples), and serum iron levels were measured (Fig. 7). As a result, it was revealed that the serum iron level increased transiently after FOLFIRI therapy even in the patient who had undergone tumor resection. Since a transient increase in serum iron level was also observed after tumor resection ($56.50 \pm 5.32 \mu\text{g/dl}$ before versus $239.0 \pm 18.95 \mu\text{g/dl}$ after the chemotherapy; an increase of $427.25 \pm 61.62\%$), the increased iron was unlikely derived from tumor cells but was likely derived from normal cells. Thus, it is reasonable to speculate that the increased iron in sera was mainly derived from normal cells, since the number of normal cells was much higher than that of the tumor cells in the body. However, it cannot be ruled out that iron is partially released from tumor cells into the blood during chemotherapy in cancer-bearing patients.

If the transient increase in serum iron observed in the present study can estimate the outcome of FOLFOX4 or FOLFIRI therapy, it could be used as one of the potential biomarkers for monitoring antitumor chemotherapy. In fact, our preliminary studies revealed that the efficacy of FOLFOX4 or FOLFIRI therapy is correlated with the response of serum iron. We are now planning to investigate the relationship between the changes in serum iron and the outcome of chemotherapy in a larger population of patients.

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