Increased hepcidin production impairs iron metabolism after pancreatoduodenectomy

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Abstract

Background: The duodenum is a central organ for iron absorption. The serum levels of iron metabolism-related markers, including serum iron (sFe), total iron-binding capacity (TIBC), serum ferritin (sFer), C-reactive protein (CRP), interleukin-6 (IL-6), hemoglobin (Hb), hematocrit (Ht), and serum hepcidin (sHep) were evaluated during the perioperative period in patients undergoing pancreatoduodenectomy (PD).

Methods: Twenty-one patients who had undergone PD at our department were enrolledin the study. Measurements of sFe, TIBC, sFer, and sHep were performed before surgery (Pre), and on postoperative days 3 (D3), 7 (D7), and 14 (D14).

Results: The median values of sFe on Pre, D3, D7, and D14 were 73.0, 30.0, 33.0, and 41.0 μg/ dL, respectively (P<0.05), and those of TIBC were 276.0, 160.0, 176.0, and 165.0 μg/ dL, respectively (P<0.05). CRP and IL-6 became maximal on D3. The median values of sHep on Pre, D3, D7, and D14 were 18.9, 42.9, 25.7, and 21.2 mg/ dL, respectively (P<0.05). Hb and Ht reached minimum values on D3 and remained low until D14. The median values of sFer on Pre, D3, D7, and D14 were 135, 301, 267, and 233 ng/ dL, respectively.

Conclusion: Severe inflammation caused by PD induces an increase in hepcidin

production, and hepcidin diverts iron to storage-type ferritin rather than to erythropoiesis. Iron administration intended for erythropoiesis during this period should therefore be avoided.

Body iron is strictly controlled within a narrow window to avoid overloading and deficiency. Human total stored iron is estimated to be 5 g, and about 60-70% of stored iron is in red blood cells. On a daily basis, about 1 mg of dietary iron is absorbed and about 1 mg of iron is lost in stools through detachment of enterocytes. There is no organized system for iron excretion, and most iron indispensable for metabolism is supplied from the reticuloendothelial system, where red blood cells are degraded at the end of their working life.

The duodenum is the main area of the GI tract for iron absorption. Dietary iron as Fe^{3+} is reduced to Fe^{2+} by duodenal cytochrome *b* reductase (Dcytb) on duodenal enterocytes (1). The Fe^{2+} is absorbed by the divalent metal transporter (DMT1) and moves to the basolateral membrane (2). It is then exported to the systemic circulation by ferroportin 1 (FPN1) (3). Ths is a critical step in iron absorption, and the function of FPN1 is blocked by a small peptide hormone, hepcidin. An increase in hepcidin results in inhibition of iron absorption, and vice versa.

Pancreatoduodenectomy (PD) is the standard procedure for treatment of pancreatobiliary cancer. In PD, because the whole duodenum is removed, a drastic change in iron absorption occurs. For this reason, PD has also been used as a model for studies of iron absorption. However, there have been no systematic studies of iron

absorption and metabolism during the perioperative period in patients undergoing PD. In this study, for the first time, we evaluated changes in body iron concentration and other iron-related molecules sequentially, in patients undergoing PD.

Patients and Methods

A total of 21 patients who had undergone PD at the Second Department of Surgery, Dokkyo Medical University, were enrolled. Their clinical backgrounds are shown in Table 1. There were 15 males and 6 females with mean age of 67.5 ± 8.5 years. The diagnoses included 6 cases of pancreatic cancer, 6 cases of bile duct cancer, 3 cases of intraductal papillary mucinous tumor, and others including autoimmune pancreatitis, chronic pancreatitis, and cancer of the papilla of Vater.

The patients took meals until the day before the operation, and were then maintained by hyperalimentation until postoperative days 5-7.

PD was performed using our previously reported method (4). The standard procedure for PD included regional and extensive lymph node dissection and up to 10-30% distal gastrectomy. Through this procedure, the whole duodenum was removed along with a portion of the stomach 1-3 cm in width from the pylorus ring and upper jejunum, and 10 cm in length distally from the Treitz ligament. The modified Child

procedure was selected for reconstruction in all cases. Operation time, intraoperative blood loss, and other operative data are also shown in Table 1. Postoperative complications included pancreatic juice leakage in 6 patients, secondary diabetes in 2, and others including anastomotic ulcer and fungal infection. Transfusion was performed in 2 patients, and the volumes of transfused blood were 2 and 6 units.

All patients were postoperatively managed by total parenteral nutrition until oral intake was usually started on postoperative day 7. Iron in the form of a trace element was given intravenously at 2 mg/day from the day after the operation until the start of oral intake.

Serum iron concentration (sFe), serum ferritin concentration (sFer), total iron-binding capacity (TIBC), C-reactive protein (CRP), interleukin-6 (IL-6), hemoglobin (Hb), hematocrit (Ht), and serum hepcidin concentration (sHep) were measured before surgery (Pre), and on postoperative days 3 (D3), 7 (D7), and 14 (D14). Only sFe was also measured on postoperative day 90 (D90). sFe, TIBC, and sFer were measured by SRL, Inc. (Tokyo, Japan) and sHep was measured by MCprot (Kanazawa, Japan). Data for IL-6 levels were available for only 9 patients, and determined using a Human IL-6 Quantikine ELISA kit (R&D System, Minneapolis, MN). All samples were measured in triplicate, in accordance with the manufacturer's recommendations.

Statistical analyses

Data were expressed as means ± standard deviations or median (minimum-maximum). Comparisons of values between two groups were analyzed by Mann-Whitney U test (two-sided). A probability value of $P<0.05$ was considered to indicate statistical significance.

Results

Figure 1A shows the change in the median value of sFe at each time point. The median values of sFe on Pre, D3, D7, and D14 were 73.0, 30.0, 33.0, and 41.0 μg/ dL, respectively, and those on D3, D7, and D14 were significantly lower in comparison to Pre (P= 0.0096, 0.0001, and 0.0058, respectively). Figure 1B shows the change in the median value of TIBC at each time point. The median values of TIBC on Pre, D3, D7, and D14 were 276.0, 160.0, 176.0, and 165.0μg/ dL, respectively, and those on D3, D7, and D14 were significantly lower in comparson to Pre (P= 0.0002, 0.0007, and 0.0125, respectively). At 3 months after PD, the median value of sFe was 53.0 μg/ dL. Although sFe tended to be low, the difference was not significant ($P>0.05$).

Figure 2 shows change in CRP and IL-6. The median values of CRP on Pre, D3, D7,

and D14 were 0.1, 7.7, 2.6, and 0.9, respectively, and those of IL-6 were 18, 175, 76, and 21 pg/ mL, respectively. The median values of CRP and IL-6 became maximal on D3 and then gradually decreased towards D14.

Figure 3A shows the changes in sHep in individual patients. In most patients, sHep was increased on D3 to D7, and decreased on D14. Figure 1B shows the median value of sHep at each time point. The median values of sHep on Pre, D3, D7, and D14 were 18.9, 42.9, 25.7, and 21.2 mg/dL, respectively, those on D3, D7, and D14 being significantly higher in comparison to Pre (P<0.0001, <0.0001, = 0.0183, respectively).

During this period, Hb (Fig. 4A) and Ht (Fig. 4B) remained low. The median values of Hb on D3, D7, and D14 were significantly lower in comparison to Pre (P= 0.0043, 0.0017, and 0.0039, respectively), and those of Ht on D3, D7, and D14 were significantly lower in comparison to Pre (P= 0.0042, 0.0006, and 0.0011, respectively).

Figure 5A shows the changes in sFer in individual patients. In most patients, sFer increased on D3 and did not change significantly up to D7. sFer peaked sharply on Day 3 in two patients, and on Day 7 in one patient. Figure 3B shows the median value of sFer at each time point. The median values of sFer on Pre, D3, D7, and D14 were 135, 301, 267, and 233 ng/dL, respectively, those on D3 and D7 being significantly higher in comparison to Pre (P= 0.0136 and 0.0237, respectively). There was no significant

difference in sFer between Pre and D14 (P= 0.1067). There was some discrepancy between Hb and Ht, and sFer: although sFer increased during D3 to D7, Hb and Ht remained low.

Discussion

PD is one of the most invasive gastrointestinal operations, with an operative mortality of 4% (5, 6). The incidence of postoperative morbidity, such as delayed gastric emptying and pancreatic fistula, may be as high as 50% (6). Because the duodenum is the central area of the GI tract for iron absorption, removal of the whole duodenum by PD has a marked effect on iron metabolism.

Previous studies have reported that patients who have undergone gastric bypass for treatment of morbid obesity, whereby the duodenum is excluded from digestive tract continuity, have lower sFe and hemoglobin concentrations (7). However, another long-term study reported that there was no iron deficiency after duodenal switch in bariatric surgery (8). These results indicate that other parts of the intestine can compensate for iron absorption after exclusion of the duodenum. In the present study, the levels of sFe and TIBC were decreased on D3 and remained low up to D14. This decrease may have been partly due to operative blood loss. One milliliter of blood

contains about 0.5 mg of iron. The median operative blood loss for the patients overall was 708 ml, indicating that about 350 mg of iron was lost. Although sFe tended to be lower 3 months after PD, the difference was not statistically significant (Fig. 1A). The main source of iron supply is not iron absorption, but release of iron from macrophages. It is estimated that, in a normal environment, 1 mg/ hour of iron is released from macrophages. Thus, during the acute phase after PD, surgical stress induces inflammation, as expressed by an increase of CRP and IL-6, and this results in an increase of hepcidin production, the hepcidin in turn suppressing the release of iron from macrophages. Three months after PD, hepcidin production was decreased, and consequently sFe was increased.

Hepcidin is a small peptide hormone produced mainly in the liver (9, 10). The function of hepcidin is to bind to FPN1, which is expressed abundantly on the cell surface of duodenal enterocytes and macrophages, and degrades FPN1. Because FPN1 is a major molecule that exports iron from the cytoplasm to the extracellular space (3), degradation of FPN1 results in retention of iron in the cytoplasm. In iron-deficiency anemia, it is known that production of hepcidin decreases, whereas in iron overloading hepcidin production increases. It is also known that inflammation stimulates hepcidin production. The mechanism of this stimulation involves inflammatory cytokines, such

as IL-6. IL-6 directly stimulates hepcidin production and leads to iron-deficiency anemia (11). Thus, an inflammatory state induces hepcidin production, and this results in disturbance of iron absorption in the duodenum and retention of iron in macrophages, leading in turn to impairment of tissue iron metabolism.

In this study, most patients showed an increase of CRP and IL-6 on D3 or D7, due to the severe surgical stress of PD, and sHep also increased during this period. At the same time, sFe and TIBC remained at levels lower than before surgery. As discussed earlier, the decrease in sFe and TIBC might have been partly caused by blood loss during the operation, but also could have been due to impairment of iron metabolism due to increased hepcidin production. In the meantime, sFer increased during this period (Fig. 5). Although the level of sFer usually parallels that of sFe, dissociation of the two parameters is sometimes observed in patients with cancer, chronic inflammation, and invasive stress (12). After PD, Hb and Ht remained at lower levels up to day 14 (Fig. 4), despite the intravenous administration of iron, and sHep increased up until D14. An increase in sHep inhibits efflux of iron from hepatocytes and macrophages, and results in impairment of erythropoiesis (13, 14). Our results, indicating a decrease of Hb and Ht, and an increase in ferritin, suggested that increased hepcidin after the acute phase of PD diverted Fe from erythropoiesis to storage in hepatocytes and macrophages in the form

of ferritin.

It appears that in the acute phase after PD, especially within 7 days, administered iron is not properly used in tissues and instead is stored by macrophages, suggesting that iron administration is not necessary. From 7 days after surgery, sFe and TIBC increased, in parallel with the decrease in sHep. Oral intake was started on D5-D7 and the inflammatory state caused by operative stress began to be relieved around this time, leading to a decrease in hepcidin production. At 3 months after PD, the sFe level did not differ significantly in comparison with Pre. After the acute phase of PD, other parts of the intestines may compensate for iron absorption after exclusion of the duodenum from digestive tract continuity, thus partly contributing to the increase in sFe.

In conclusion, increased hepcidin production was shown to impair iron metabolism after PD. Our findings indicate that aggressively administered iron may not be properly used by tissues, and should be avoided until 7 days after surgery.

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Figure legends

Figure 1 Changes in serum iron (sFe) and TIBC

(A) The median values of sFe on D3, D7, and D14 were significantly lower than that of Pre (*P*= 0.0096, 0.0001, and 0.0058, respectively). **SS**: statistically significant. Horizontal bar represents median value.

(B)The median values of TIBC on D3, D7, and D14 were significantly lower than that of Pre (*P*= 0.0002, 0.0007, and 0.0125, respectively). **SS**: statistically significant. Horizontal bar represents median value.

Figure 2 Changes in CRP and IL-6

(A) The median values of CRP on D3 and D7 were significantly lower than that of Pre (*P*<0.0001 and <0.0001, respectively). **SS**: statistically significant. Horizontal bar represents median value.

(B) The median values of IL-6 on D3, D7, and D14 were significantly lower than that of Pre (*P*<0.0001 and <0.0001, respectively). **SS**: statistically significant. Horizontal bar represents median value.

Figure 3 Changes in serum hepcidin (sHep)

(A) Changes in sHep in individual patients. (B) The median values of sHep on D3, D7, and D14 were significantly higher than that of Pre ($P < 0.0001$, <0.0001, and = 0.0181, respectively). Horizontal bar represents median value.

Figure 4 Changes in Hb and Ht

(A) Changes in Hb in individual patients. The median values of Hb on D3, D7, and D14 were significantly lower than that of Pre ($P = 0.0043$, 0.0017, and 0.0039, respectively). **SS**: statistically significant. Horizontal bar represents median value.

(B) Changes in Ht in individual patients. The median values of Ht on D3 and D7 were significantly higher than that of Pre ($P = 0.0043$, 0.0017, and 0.0039, respectively). SS: statistically significant. Horizontal bar represents median value.

Figure 5 Change in sFer

(A) Changes in sFer in individual patients. (B) The median values of sFer on D3 and D7 were significantly higher than that of Pre (*P*= 0.0136 and 0.0237, respectively). There was no significant difference in sFer between Pre and D14 (*P*= 0.1067). **SS**: statistically significant. Horizontal bar represents median value.