Preoperative blood management in colorectal cancer surgery: the controversial role of iron

Wilson, M.J.

Chapter 6 // **Iron therapy as treatment of anemia: a potentially detrimental and hazardous strategy in colorectal cancer patients**

M.J. Wilson, J.J. Harlaar, J. Jeekel, M. Schipperus, J.J. Zwaginga

ABSTRACT

In colorectal cancer patients, iron therapy, and especially intravenous iron therapy, is increasingly used to treat anemia and reduce the use of blood transfusions. However, iron has also been shown to be an essential nutrient for rapidly proliferating tissues and cells. In this respect, anemia of inflammation, characterized by limited duodenal iron uptake and sequestration of iron into the reticuloendothelial system, might be regarded as a potentially effective defense strategy of the human body against tumor growth. We therefore hypothesize that iron therapy, by supporting colorectal tumor growth and increasing the metastatic potential, may worsen tumor prognosis in colorectal cancer patients. This hypothesis is particularly supported for colorectal cancer by laboratory, epidemiological and animal studies, demonstrating the role of iron in all aspects of tumor development growth. Compared to non-malignant colon cells, tumor cells differ in the levels and activity of many iron import and export proteins, resulting in an increase in intracellular iron level and enhanced proliferation. In addition, it is demonstrated that iron is able to amplify Wnt signaling in tumors with Apc mutation, a critical mutation in the development of colorectal cancer. If our hypothesis is to be confirmed, current practice of iron administration, as treatment for anemia and as replacement of blood transfusions, can be hazardous and should be completely reconsidered.

INTRODUCTION

Colorectal cancer is the third most commonly diagnosed cancer in men and the second in women worldwide, accounting for more than 1.4 million new cases and 694 000 associated deaths worldwide.[1] Anemia (hemoglobin <12.0 g/dL) is the most frequent hematological manifestation in patients with cancer, occurring in >40% of the cases. In colorectal cancer, anemia is even reported in around 60% of the cases.[2] This anemia is most often associated with iron deficiency,[3] but more importantly also with impaired disease-free and overall survival in cancer patients.[4, 5]

As both blood transfusions and erythropoiesis-stimulating agents (ESAs) are, similar to anemia, independently associated with an increased risk of colorectal cancer recurrence and increased mortality,[6-9] the use of iron to reverse anemia has gained more attention. In this regard, while oral iron does correct anemia, it also causes constipation, and is largely ineffective in patients with anemia of inflammation, characterized by reduced duodenal iron uptake and iron mobilization from the reticulo-endothelial system. In comparison with oral iron, intravenous iron does not have these disadvantages and is therefore more and more preferred.[10] In colorectal cancer patients, several cohort studies have shown that intravenous iron therapy indeed optimizes preoperative hemoglobin level. A net reduction of blood transfusions by intravenous iron, however, is not conclusively shown as of yet. [11-14]

In contrast to the short-term effect of iron therapy to increase the hemoglobin level, strikingly, possible long-term effects in colorectal cancer patients, such as survival, are so far hardly studied. These long-term effects are of special interest since anemia of inflammation is believed to be a potentially defense strategy of the human body to limit the growth of tumor cells.[15] In this respect, the results of laboratory, epidemiological and animal studies indeed have shown iron's role in all aspects of cancer development and cancer growth.[11, 16-21] Finally, corroborating evidence implicates that especially gastrointestinal cancer cells, likely by their original iron-absorbing nature, have an altered iron homeostasis.[22]

THE HYPOTHESIS

We hypothesize that iron therapy may worsen colorectal tumor prognosis by supporting colorectal tumor growth and increasing the metastatic potential. Although no direct evidence is available to date, accumulative data from experimental studies are in favor of this hypothesis. In this respect, importantly, iron therapy is increasingly used with the aim of optimizing hemoglobin level and reducing the need for blood transfusions. Therefore, this hypothesis is in striking contrast with current practice in patient blood management. We evaluated the current evidence supporting this hypothesis in the following part.

EVALUATION OF THE HYPOTHESIS

Iron is an essential nutrient participating in numerous biological and cellular processes such as hemoglobin-mediated oxygen transport, DNA synthesis and cell proliferation and growth. As mammals do not possess any regulated mechanisms for iron excretion from the body, iron metabolism is maintained by the tight control of dietary iron absorption in the duodenum. Intracellular iron transport is mainly controlled by three iron transport proteins: 1. divalent metal transporter 1 (DMT1), facilitating the transport of dietary iron across the apical membrane of enterocytes 2. ferroportin, facilitating the export across the basolateral membrane into the bloodstream, and 3. transferrin receptor 1 (TfR1), facilitating the import across the basolateral membrane into the cell. Systemically, iron homeostasis is regulated by hepcidin, which is produced by hepatocytes and inhibits the release of iron from enterocytes and macrophages into the circulation by inducing the internalization and subsequent degradation of ferroportin.[23, 24] The level of hepcidin is controlled by many factors including iron stores, hypoxia, anemia and erythropoiesis.[25, 26] Whereas iron deficiency, enhanced red blood cell production, and hypoxia decrease hepcidin expression to accelerate iron absorption, iron overload and inflammatory stimuli like IL-6 induce increased hepcidin expression. The latter is the cause of a hepcidin-mediated decrease in iron uptake and utilization, so called anemia of chronic disease or anemia of inflammation.

Abovementioned background information on the normal regulation of iron metabolism is essential to put the modifications in intracellular iron regulation in colorectal cancer cells in perspective. In distinct favor of our hypothesis, many transport proteins that were originally studied for their roles in normal iron metabolism have now been shown to contribute to malignant tumor growth. Compared to non-malignant colon cells, iron import proteins, such as DMT1 and TfR1, are upregulated, while ferroportin, the only known iron export protein, is downregulated in colon tumor cells, subverting the normal homeostatic control into a chronic iron acquisition state enabling enhanced proliferation.[27-29] More in detail, the presence of the key intestinal tumor suppressor Apc seems to play a pivotal role. The Apc gene is the most commonly mutated tumor suppressor gene in sporadic colorectal cancer,[30] and it is shown that especially Apc-deficient (i.e. mutant Apc) cells appear critically dependent iron for efficient tumor growth. In Apc-deficient cells, raising the levels of iron induces the expression of TfR1 and DMT1, resulting in increased iron content in the cells and increased proliferation, while removal of iron drives apoptosis of Apc-deficient cells. This is the exact opposite to what is observed in colorectal cancer cells with wildtype Apc.[17, 31] In addition, it is demonstrated that, in mouse models, the growth rate of tumor xenografts is increased by high levels of dietary iron.[32, 33]

Finally, in studying the proliferative effect of iron in colorectal tumors, a clear link between iron and Wnt signaling was found[30, 34, 35]. Wnt signaling plays a critical role in regulating homeostasis and self-renewal of tissues, and in the intestinal epithelium it promotes proliferation and differentiation of stem cells in the intestinal crypts. Aberrant Wnt signaling is closely related to a mutation

in Apc, and is an important hallmark for colorectal cancer development. Importantly, and supporting our hypothesis, it is demonstrated that iron, in the background of an Apc mutation, is able to amplify Wnt signaling, and with it induction of cell growth.[36] Therefore, in the presence of an Apc mutation, iron will affect Wnt signaling and with it an increase in the tumorigenic and metastatic potential.[37]

CONSEQUENCES OF THE HYPOTHESIS AND DISCUSSION

Substantial evidence suggests that iron promotes colorectal tumor growth and potentially increases the metastatic potential of colorectal tumor cells. Therefore, the legitimate question arises as to whether the use of iron therapy, either orally or intravenously, is a safe and optimal treatment strategy in anemic colorectal cancer patients.

We hypothesize that iron in general (i.e. both oral and intravenous) may worsen colorectal tumor prognosis, but the different routes of administration should be considered. In colorectal cancer patients, oral iron often is poorly absorbed. As a consequence, only a fraction of the dietary (i.e. orally administered) iron will reach the sites of erythropoiesis and a significant part will reach the site of the primary tumor. At the site of the primary tumor, the oral iron will be able to affect Wnt signaling and contribute to enhanced tumor growth and increased metastatic potential. However, and in contrast with intravenous iron, the effect of oral iron will be limited to the primary tumor. In this respect, intravenous iron might have more influence if metastases are present.

In addition to the different routes of administration, we hypothesize that the assessment of type of iron deficiency anemia might be important in studying the long-term effect of iron therapy. Chronic blood loss, as can be envisioned by bleeding from gastrointestinal tumors, in this respect causes absolute iron deficiency (AID), characterized by depleted iron stores. Functional iron deficiency (FID), in contrast, is caused by impaired iron homeostasis and is, due to increased hepcidin production, characterized by reduced iron uptake from the duodenum and iron mobilization from the reticulo-endothelial system. FID resulting in anemia is also known as anemia of inflammation or anemia of chronic disease. Despite definite evidence, FID could be regarded as a potentially effective defense strategy to inhibit growth of pathogens and tumor cells.[15] As, in the event of FID, a large fraction of oral iron will, due to poor absorption in the duodenum, reach the site of the primary tumor, we hypothesize that iron supplementation will be more hazardous in patients with FID, as compared to patients with AID.

In assessing the effect of iron therapy on tumor growth and tumor prognosis, the dose-response relationship will be particularly important. A single dose of intravenous iron normally contains 1000 mg, which is, as compared to a daily iron uptake of 1-2 mg, an extremely high amount of extra iron. Total body iron is 3-4 g, and this quantity is tightly regulated. However, the body has no mechanism to excrete excess iron and only less than 0.1% of total iron is lost on average daily,

mostly through urine, sweat and feces. Therefore, and despite the relatively large amount of 3-4 g of total body iron, we hypothesize that a single dose of intravenous iron or continuous supplementation of oral iron (i.e. normally 400-500 mg daily) could already affect tumor growth and therefore be harmful, especially in iron-dependent tumors.

Testing our hypothesis will be challenging. In a retrospective cohort study significant differences between an iron-treatment and non-iron treatment group (e.g. baseline hemoglobin levels and use of blood transfusions) could, despite possible correction by multivariable regressions analyses, potentially indicate selection bias and have significant impact on the outcome. Therefore, to show the validity of our hypothesis, the clinical long-term effect of iron therapy should be studied in a prospective trial. In such a trial, anemic patients should be randomized into an iron-treatment and a non-treatment group. In addition, clinical outcome should be correlated with both tumor (e.g. expression levels of iron transporters, Apc status, tumor stage and molecular subtype[38]) and patient characteristics. Only such a set up will allow identification of iron-dependent tumor growth and potential high-risk patients. Apart from studying the clinical long-term consequences of iron therapy, assessing the optimal management of anemia in colorectal cancer patients is more challenging. For this purpose, the detrimental long-term effects of iron treatment must be compared with those of not only anemia, but also alternatives to treat anemia like ESAs and more important blood transfusions. The problem here is that a head-to-head comparison of blood transfusion and iron therapy seems almost impossible. The indications for both therapies are namely clearly different. Iron therapy is indicated in patients with a mild to moderate anemia, while blood transfusion are only administered in case of severe anemia. As a useful alternative for clinically assessing the optimal management of anemia, animal experiments could be considered. In an ortho- or heterotopic rodent model, the effect on tumor growth of both anemia, blood transfusion and iron therapy (both oral and intravenous) could be accurately assessed and compared.

In conducting a prospective trial randomizing anemic patients in an iron-treatment and a non-treatment group, ethical issues should be considered. Anemia itself is considered as a major risk factor for impaired disease-free and overall survival in cancer patients,4, 5 and therefore the inclusion of a non-treatment (i.e. anemic) group could be considered unethical. To challenge this ethical issue, the proposed and abovementioned animal experiments studying the effect on tumor growth of both anemia, blood transfusion and iron therapy will play a pivotal role. These animal experiments should precede a clinical trial studying the long-term effects of iron therapy. If anemia is proven to be significantly associated with highest tumor growth, the clinical study design should be reconsidered. This could, for example, include the administration of iron therapy in all anemic patients, subdivided into patients with iron-dependent and non-iron dependent colorectal tumor phenotypes. Assessment of the iron-dependency of the tumor could be done by identification of gene and protein expression levels of iron transporters. If iron therapy is proven

to be significantly associated with impaired tumor prognosis in the iron-dependent group, this probably indicates the hazardous effect of iron therapy.

Interestingly, corroborating data about the possible detrimental effects of iron can be deduced from a report by Harlaar et al,[39] comparing the long-term effects of autologous and allogeneic blood transfusions in colorectal cancer patients. They unexpectedly showed that patients with autologous blood transfusion, with an a priori lower probability to dysregulate the immune system of the recipient, showed an inferior long-term survival as compared to patients transfused with allogeneic blood. Interestingly, the patients with autologous blood transfusion additionally received preoperative oral iron therapy to maintain normal hemoglobin levels after blood donation.

In conclusion, if iron therapy indeed can be shown to worsen colorectal tumor prognosis, this should change current management of anemia in colorectal cancer patients. Focus will be shifted, and minimization of the use of blood transfusions will no longer be the main objective. Paradigms will be shifted in patient blood management, which will bear major changes in oncological care as a whole.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; 136(5):E359-86.
2. Ludwig H, Van Belle S, Barrett-Lee P, et al. The European Cancer AnemiaSurvey (ECAS): a large, multinational, prospective survey defining the prevalence, incidence, and treatment of anemiain cancer patients. *Eur J Cancer* 2004; 40(15):2293-306.
3. Wilson MJ, Dekker JWT, Harlaar JJ, et al. The role of preoperative iron deficiency in colorectal cancer patients: prevalence and treatment. *Int J Colorectal Dis* 2017.
4. Caro JJ, Salas M, Ward A, et al. Anemia as an independent prognostic factor for survival in patients with cancer: a systemic, quantitative review. *Cancer* 2001; 91(12):2214-21.
5. Wilson MJ, van Haaren M, Harlaar JJ, et al. Long-term prognostic value of preoperative anemia in patients with colorectal cancer: A systematic review and meta-analysis. *Surg Oncol* 2017; 26(1):96-104.
6. Acheson AG, Brookes MJ, Spahn DR. Effects of allogeneic red blood cell transfusions on clinical outcomes in patients undergoing colorectal cancer surgery: a systematic review and meta-analysis. *Ann Surg* 2012; 256(2):235-44.
7. Amato A, Pescatori M. Perioperative blood transfusions for the recurrence of colorectal cancer. *Cochrane Database Syst Rev* 2006(1):CD005033.
8. Bohlius J, Schmidlin K, Brillant C, et al. Recombinant human erythropoiesis-stimulating agents and mortality in patients with cancer: a meta-analysis of randomised trials. *Lancet* 2009; 373(9674):1532-42.
9. Pascual M, Bohle B, Alonso S, et al. Preoperative administration of erythropoietin stimulates tumor recurrence after surgical excision of colon cancer in mice by a vascular endothelial growth factor-independent mechanism. *J Surg Res* 2013; 183(1):270-7.
10. Borstlap WA, Buskens CJ, Tytgat KM, et al. Multicentre randomized controlled trial comparing ferric(III)carboxymaltose infusion with oral iron supplementation in the treatment of preoperative anemiain colorectal cancer patients. *BMC Surg* 2015; 15:78.
11. Borstlap W, Stellingwerf ME, Moolla Z, et al. Iron therapy for the treatment of preoperative anemiain patients with colorectal carcinoma: a systematic review. *Colorectal Dis* 2015.
12. Calleja JL, Delgado S, del Val A, et al. Ferric carboxymaltose reduces transfusions and hospital stay in patients with colon cancer and anemia. *Int J Colorectal Dis* 2016; 31(3):543-51.
13. Keeler BD, Simpson JA, Ng O, et al. Randomized clinical trial of preoperative oral versus intravenous iron in anemic patients with colorectal cancer. *Br J Surg* 2017.
14. Laso-Morales M, Jerico C, Gomez-Ramirez S, et al. Preoperative management of colorectal cancer-induced iron deficiency anemia in clinical practice: data from a large observational cohort. *Transfusion* 2017.
15. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005; 352(10):1011-23.
16. Fonseca-Nunes A, Jakszyn P, Agudo A. Iron and cancer risk--a systematic review and meta-analysis of the epidemiological evidence. *Cancer Epidemiol Biomarkers Prev* 2014; 23(1):12-31.
17. Ilsley JN, Belinsky GS, Guda K, et al. Dietary iron promotes azoxymethane-induced colon tumors in mice. *Nutr Cancer* 2004; 49(2):162-9.
18. Stevens RG, Jones DY, Micozzi MS, et al. Body iron stores and the risk of cancer. *N Engl J Med* 1988; 319(16):1047-52.
19. Torti SV, Torti FM. Iron and cancer: more ore to be mined. *Nat Rev Cancer* 2013; 13(5):342-55.
20. Xue X, Shah YM. Intestinal iron homeostasis and colon tumorigenesis. *Nutrients* 2013; 5(7):2333-51.
21. Zhang C, Zhang F. Iron homeostasis and tumorigenesis: molecular mechanisms and therapeutic opportunities. *Protein Cell* 2015; 6(2):88-100.
22. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144(5):646-74.
23. Nemeth E, Tuttle MS, Powelson J, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004; 306(5704):2090-3.

24. Wu XN, Su D, Wang L, et al. Roles of the hepcidin-ferroportin axis and iron in cancer. *Eur J Cancer Prev* 2014; 23(2):122-33.
25. Ganz T, Olbina G, Girelli D, et al. Immunoassay for human serum hepcidin. *Blood* 2008; 112(10):4292-7.
26. Nicolas G, Chauvet C, Viatte L, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. *J Clin Invest* 2002; 110(7):1037-44.
27. Brookes MJ, Hughes S, Turner FE, et al. Modulation of iron transport proteins in human colorectal carcinogenesis. *Gut* 2006; 55(10):1449-60.
28. Hamara K, Bielecka-Kowalska A, Przybylowska-Sygut K, et al. Alterations in expression profile of iron-related genes in colorectal cancer. *Mol Biol Rep* 2013; 40(10):5573-85.
29. Ward DG, Roberts K, Brookes MJ, et al. Increased hepcidin expression in colorectal carcinogenesis. *World J Gastroenterol* 2008; 14(9):1339-45.
30. Bienz M, Clevers H. Linking colorectal cancer to Wnt signaling. *Cell* 2000; 103(2):311-20.
31. Radulescu S, Brookes MJ, Salgueiro P, et al. Luminal iron levels govern intestinal tumorigenesis after Apc loss in vivo. *Cell Rep* 2012; 2(2):270-82.
32. Hann HW, Stahlhut MW, Blumberg BS. Iron nutrition and tumor growth: decreased tumor growth in iron-deficient mice. *Cancer Re*s 1988; 48(15):4168-70.
33. Hann HW, Stahlhut MW, Menduke H. Iron enhances tumor growth. Observation on spontaneous mammary tumors in mice. *Cancer 1991; 68(11):2407-10.*
34. Klaus A, Birchmeier W. Wnt signalling and its impact on development and cancer. Nat Rev Cancer 2008; 8(5):387-98.
35. Reya T, Clevers H. Wnt signalling in stem cells and cancer. *Nature* 2005; 434(7035):843-50.
36. Brookes MJ, Boult J, Roberts K, et al. A role for iron in Wnt signalling. *Oncogene* 2008; 27(7):966-75.
37. Vermeulen L, De Sousa EMF, van der Heijden M, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol* 2010; 12(5):468-76.
38. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. Nat Med 2015.
39. Harlaar JJ, Gosselink MP, Hop WC, et al. Blood transfusions and prognosis in colorectal cancer: long-term results of a randomized controlled trial. *Ann Surg* 2012; 256(5):681-6; discussion 686-7.