CANCER BIOTHERAPY & RADIOPHARMACEUTICALS
Volume 21, Number 6, 2006

06-364

Uptake of Anti-Anemic Substance Ferric-Sorbitol-Citrate by Normal and Malignant Cells and Its Effects on Expression of Transferrin Receptor 1 and Ferritin

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ABSTRACT

Iron-containing antianemic drug ferric-sorbitol-citrate (FSC) inhibits the proliferation of various cancer cell lines in vitro *and causes a regression of experimental murine tumors* in vivo *but does not affect the proliferation of nonmalignant cells. Growth modification caused by FSC iron involves a diminished expression of Bcl-2 and an overexpression of p53 proto-oncogene, accompanied by an increased incidence of apoptosis. Aiming to evaluate further the activity principle of the anticancer effects of this antianemic drug, in this study, we analyzed the utilization of iron from FSC and the effects of FSC iron on transferrin receptor 1 (TfR1) and ferritin expression. Without FSC iron, all the cell lines had an equal expression of TfR1, but if cultured in FSC-supplemented medium, human colon SW620 and laryngeal carcinoma Hep cells exhibited a lower expression of TfR1-positive cells than nonmalignant Wi38 fibroblasts and pancreatic carcinoma MiaPaCa2 cells. The most sensitive to FSC iron were colon carcinoma SW620 cells, whereas Wi38 fibroblasts were not sensitive at all. Increased iron uptake by colon carcinoma cells was noticed in the first 3 hours of the incubation with FSC iron, whereas higher FSC iron concentrations and longer incubation also impaired ferritin expression in SW260 colon carcinoma cells. Thus, the anticancer ability of FSC could result from its higher initial utilization of iron and consecutive negative signal for the expression of TfR1 in tumor cells. Tumor cells containing lower amounts of ferritin are probably more sensitive to oxidative stress caused by iron overload, whereas FSC iron itself was proven to be chemically stable and did not induce lipid peroxidation.*

Key words: ferric-sorbitol-citrate, iron metabolism, transferrin receptor 1, ferritin, lipid peroxidation, carcinoma cells

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INTRODUCTION

Iron is essential for cellular metabolism, viability, and growth regulation. On the other hand, free iron is pro-oxidant and can, therefore, damage

cells owing to the attack of reactive oxygen species and lipid peroxidation products on bioactive macromolecules, such as proteins and nucleic acids.[1] Therefore, iron metabolism within cells is regulated by proteins responsible for iron uptake (transferrin, transferrin receptor) and storage (ferritin).[2] The level of transferrin receptor 1 (TfR1) is related to iron requirements associated with cell proliferation. Hence, a high expression of TfR1 occurs in a variety of cancer tissues.[3] Intracellular iron is stored and stabilized in ferritin complexes, which prevents the formation of toxic free radical species that would otherwise cause cellular damage.[4]

Disruption of cellular iron homeostasis by antitransferrin receptor antibodies or iron chelators inhibits the growth of malignant cells *in vitro* and *in vivo*.[5,6]

As we have previously shown, iron in the form of ferric-sorbitol-citrate (FCS) (Jectofer; Astra, Linz, Austria) inhibited the proliferation of CaCo2 (colon carcinoma), Hep2 (laryngeal carcinoma), MiaPaCa2 (pancreatic carcinoma), and HeLa (cervix carcinoma) cell lines but did not affect the proliferation of the nonmalignant fibroblast cell line HEF522 and human bone marrow cells.[7,8] FSC also caused a regression of B16 mouse melanoma *in vitro* and *in vivo*.[9] An increased number of cells in G1 and in early S-phase suggested that iron excess blocked the cell cycle before the onset of DNA synthesis.[10] Because cells without iron are unable to proceed from G1 to the S-phase of the cell cycle,[11] we supposed that the antitumor effects of FSC involved the uptake and intracellular metabolism of iron. Therefore, we evaluated the expression of TfR1 and the ferritin content in different types of tumor cells incubated with FSC *in vitro* and measured iron concentration in culture medium at different time intervals. The possibility that FSC induced lipid peroxidation was explored in experimental models containing unsaturated fatty acids or lyophilized human plasma.

MATERIALS AND METHODS

Ferric-Sorbitol-Citrate Complex

FSC in the form of an antianemic drug, Jectofer (Astra, Linz, Austria), was used. The drug was kept in the dark at room temperature before use. For experimental purposes, it was dissolved in freshly prepared Dulbecco's modified Eagle's cell culture medium (DMEM) (Sigma Chemical Co., St. Louis, MO). Final concentrations of the FSC iron were from 10^{-5} to 10^{-3} M. These concentration ranges were chosen on the basis of previous experience with the ability of FSC iron to modify tumor cell growth *in vitro*.[7,8]

Cells and Culture Conditions

Cell lines of human colon carcinoma (SW620), laryngeal carcinoma (Hep2), and pancreatic carcinoma (MiaPaCa2) were used. The human fibroblast cell line (Wi38) served as the nonmalignant control. The cells were stored in liquid nitrogen. Thawed cells were cultured in DMEM, supplemented with 10% of fetal calf serum (FCS) at 37°C in a humidified air atmosphere with 5% CO_2. The number of viable cells was determined by means of trypan blue exclusion and 300-μL aliquots of the cell suspension containing 5×10^5 cells/mL were seeded into chamber slides (Nalge Nunc International; Naperville, IL). After 24 hours, the medium (DMEM supplemented with 10% FCS) was discarded, and fresh medium containing FSC was added. The final concentrations of iron in medium were 10^{-5} to 10^{-3} M. Cell cultures with or without iron were then incubated for 3, 24, 48, or 72 hours.

Immunocytochemistry

For immunostaining, cell smears were blocked for endogenous peroxidase activity, using 3% hydrogen peroxide in methanol. Monoclonal mouse antihuman TfR1 antibody (CD 71; Dako, Glostrup, Denmark) and peroxidase-conjugated rabbit antihuman ferritin antibody (Dako) were used as the primary antibodies. They were diluted at 1:20 (TfR1) or 1:150 (ferritin antibody) and incubated at +4°C overnight. A secondary antibody (rabbit-antimouse antibody) was diluted in phosphate buffered saline (PBS; 1:10) and incubated for 1 hour. The 3,3′-diaminobenzidine in the PBS solution (0.0025%) was used as chromogen. The sections were counterstained with hematoxylin.[12,13]

One hundred (100) cells per sample were counted at three different spots of the smear, and the results were expressed as the mean percentage ± standard deviation and as median percentage (range, min–max) of positive cells containing transferrin receptors or ferritin.

Measurement of Iron Concentration in Culture Medium

Media in which the cells were cultured for 3, 24, 48, or 72 hours were analyzed for the iron con-

centration in triplicates. One (1) mL of each sample was weighed, diluted to 100 mL with double-distilled water, and adjusted to a pH of 3 with the addition of hydrochloric acid or ammonium hydroxide, as needed. After the pH adjustment, 2 mL of 1% (w/v) ammonium-pyrolidinedithiocarbamate (APDC) were added into each flask. Complexation was allowed for 20 minutes, and the suspension was filtered through a Millipore HAWP filter (pore size 0.45 μm, diameter 25 mm). Prepared thin targets were air dried, protected by a thin mylar foil (2 μm), inserted into a plastic carrier, placed above a ^{109}Cd X-ray source of the X-ray spectrometer at an angle of 49.76°, and irradiated for 10,000 seconds. These thin targets were analyzed by energy dispersive X-ray fluorescence (EDXRF). The X-ray radiation from the sample was recorded by means of a Si (Li) detector (Canberra; Meriden, CT) cooled with liquid nitrogen. Detector size was 30 mm^2, Si thickness 3 mm, Be window 25 μM, and FWHM for 5.9 KeV ^{55}Fe was 165 eV. The angle between the sample and the detector was 74.05° and the distance was 2.5 cm. The spectra were collected by means of Genie—2000 software (Canberra). Spectral data were analyzed by WinAxil software (Canberra). The system was calibrated by using different concentrations of Fe (III) (Merck) with the addition of DMEM medium that was used in the experiment *in vitro*. Iron concentrations were calculated with "Compared method" from the WinFund package (Canberra).

AU1

AU2

Determination of Lipid Hydroperoxides

The possible ability of FSC iron to cause lipid peroxidation was determined using lyophilized human blood plasma as a substrate (Institute of Immunology; Zagreb, Croatia), containing 13% (w/w) lipid, as the representative natural mixture of oleic: 26.4%; linoleic: 21.6%; linolenic: 1.0% (w/w).

The samples were mixed with the appropriate amount of FSC iron (10^{-3} M, which is equivalent to 9×10^{-3} mol dm^{-3} Fe^{III}, and 10^{-5} M, which is equivalent to 0.09×10^{-3} mol dm^{-3} Fe^{III}) dissolved in redistilled water, and left in the dark at approximately 37°C under air atmosphere. Control samples were prepared without FSC. To assure that the possible pro-oxidative capacity of FSC iron would be detected, the samples were incubated for 24, 48, or 72 hours.

Lipid hydroperoxides (LOOH) formed by autoxidation were determined by spectrophotometry of the ferric thiocyanate complex. This complex was formed on the oxidation of Fe^{II} iron by hydroperoxides and subsequent complexing of Fe^{III} irons with thiocyanate, which yields an intense red coloration.[14,15] Absorbance at 500 nm was measured against the different blank sample, which had the same composition, except for the reagent solution. The concentrations of LOOH were calculated using the molar absorptivity of the ferric thiocyanate complex determined: 58 440 dm^3 mol^{-1} cm^{-1}.[16] Details of the method, its modification, and procedure were given in the paper.[16]

Extraction of the lipid phase from the samples of lyophilized human blood plasma was carried out with deaerated CH_2Cl_2–MeOH (2:1, v/v). The appropriate amounts of deaerated CH_2Cl_2–MeOH (2:1, v/v), deaerated extract, and reagent solution were mixed in a volumetric flask. The total amount of the lipids did not exceed 2.6 mg/mL; thus, the sensitivity of the described method was achieved.

Beside the ferric thiocyanate method, the lipid peroxidation of the unsaturated fatty acids was determined by recording the absorption of the conjugated dienes. The analyzed samples were prepared by dissolving aliquots of extract of peroxidized linoleic acid in deaerated MeOH. The absorbance at 234 nm was determined against a methanol blank. The amount of hydroperoxides produced was calculated using the molar absorptivity determined by a standard solution of 13-hydroperoxy linoleic acid in MeOH: ε(234 nm) = (28 950 ± 1 300) dm^3 mol^{-1} cm^{-1}.

Statistics

The Mann-Whitney test was used to determine the significance of the difference between two independent samples. The Kruskal-Wallis test was used to determine whether or not the values of a particular variable differed between three or more groups. *p*-values below 0.05 were considered statistically significant.

RESULTS

TfR1 expression

TfR1 were equally expressed by normal human fibroblasts and by the cells of human pancreatic, colon, and laryngeal carcinoma cell lines (Fig. 1). Incubation for 3 hours with 10^{-4} M FSC iron diminished TfR1 expression in colon (SW620) and

F1

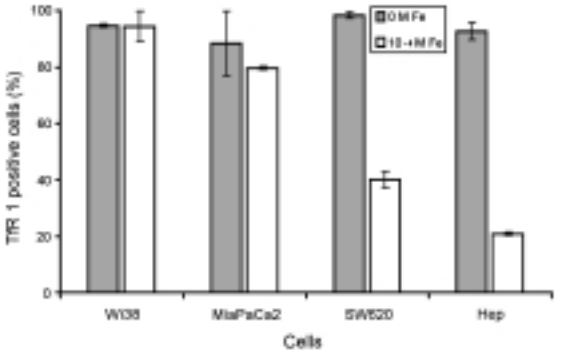

Figure 1. The effect of ferric-sorbitol-citrate (FSC) on the expression of transferrin receptor 1 on fibroblast cells (Wi38), pancreatic carcinoma cells (MiaPaCa2), colon carcinoma cells (SW620), and laryngeal carcinoma cells (Hep). The cells were incubated for 3 hours in medium and then treated with FSC iron (10^{-4} M) for 3 hours.

laryngeal carcinoma (Hep2) cells from 98.3 to 40.0 ($p < 0.05$) and from 92.7 to 20.7 ($p < 0.05$), respectively. Pancreatic carcinoma cells (MiaPaCa2) and normal fibroblasts (Wi38), however, preserved a high expression of TfR1 in the presence of iron. At the beginning of the incubation, 88.3 TfR1 positive pancreatic carcinoma cells and 94.7 TfR1 positive normal fibroblasts were determined, whereas after incubation with FSC iron, there were 79.7 TfR1 positive pancreatic carcinoma and 94.3 TfR1 positive fibroblasts ($p = 0.827$ and 0.507, respectively) (Fig. 1).

Because human colon carcinoma SW620 cells have shown medium sensitivity to FSC iron treatment from the three carcinoma cell lines tested, we used colon carcinoma cells to test further effects of different concentrations of FSC iron and extended treatment on malignant cells in comparison to the nonmalignant Wi38 fibroblasts (Fig. 2). Longer incubation with FSC iron (for 24 hours) or higher concentration (10^{-3} M) diminished TfR1 expression in SW620 colon carcinoma cells even more. Hence, there were only 1.7 TfR1 positive colon carcinoma cells ($p < 0.05$) after 24 hours of FSC iron treatment with 10^{-3} M or 10^{-4} M FSC iron. On the contrary, the same treatment with FSC iron of Wi38 fibroblasts indicated a tendency of a rise of TfR1 in nonmalignant cells, but this was not significant ($p = 0.507$).

F2

Ferritin Content

In medium without FSC, after 3 hours of incubation, ferritin was expressed in 99.7 of Wi38 fibroblasts and in 80.3 of SW620 colon carcinoma cells. After incubation for 24 hours, a decrease of the intracellular ferritin content to 56.7 in fibroblasts and to 32.0 in colon carcinoma cells was observed (for both, $p < 0.05$) (Fig. 3).

F3

During the initial 3 hours of incubation, FSC decreased ferritin expression in Wi38 fibroblasts. Thus, in medium supplemented with FSC (iron concentration 10^{-5} M), there were only 13.7 ferritin-positive fibroblasts after 3 hours ($p = 0.046$). Higher concentrations of FSC (10^{-4} and 10^{-3} M) increased the percentages of ferritin-positive fibroblasts to 29.7 ($p < 0.05$) and 45 ($p < 0.05$), respectively. After 24 hours, the percentage of ferritin-positive cells in iron-supplemented cultures did not change significantly ($p = 0.645$) and there were 79.3 at an iron concentration of 10^{-5} M, 67.7 at an iron concentration of 10^{-4} M, and 74.3 at an iron concentration of 10^{-3} M ferritin-positive fibroblasts.

Colon carcinoma SW620 cells incubated in iron-supplemented medium for 3 hours contained 3–5 times more ferritin-positive cells, in comparison to the fibroblasts at all three iron concentrations ($p < 0.001$): 69.3 at an iron concentration of 10^{-5} M; 81.7 at an iron concentration

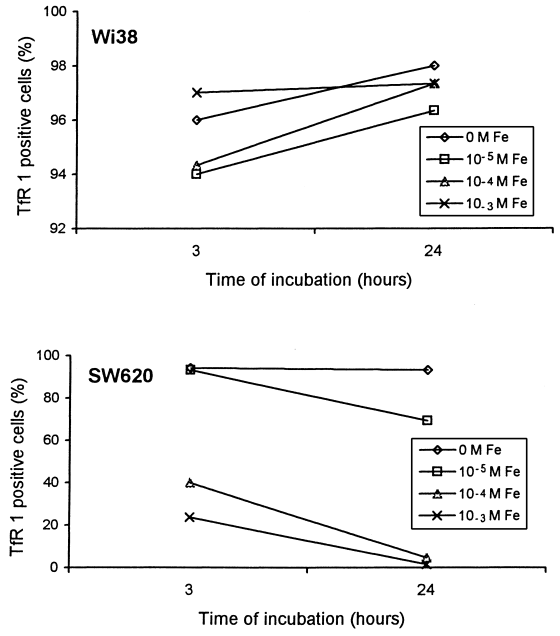

Figure 2. Impact of ferric-sorbitol-citrate (FSC) on transferrin receptor 1 expression in nonmalignant (normal) fibroblasts (Wi38) and colon carcinoma cells (SW620). Cells were incubated for 3 or 24 hours. FSC iron concentrations were from 10^{-5} to 10^{-3} M.

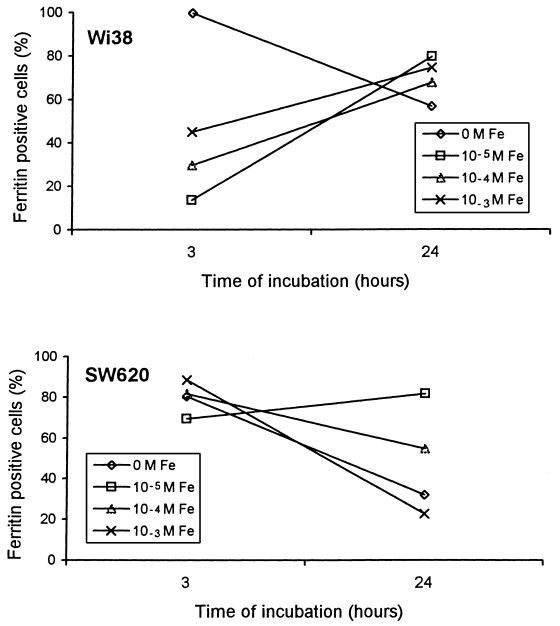

Figure 3. The percentage of ferritin-positive nonmalignant (normal) fibroblasts (Wi38) and colon carcinoma cells (SW620) after incubation with ferric-sorbitol-citrate (FSC) iron. Cells were incubated for 3 or 24 hours. FSC iron concentrations were from 10^{-5} to 10^{-3} M.

of 10^{-4} M; and 88.3 at an iron concentration of 10^{-3} M. However, incubation with FSC at an iron concentration of 10^{-4} M and 10^{-3} M for 24 hours resulted in a significant decrease of ferritin to 54.7 and 22.7, respectively, when compared to the fibroblasts incubated with the same iron concentrations ($p < 0.01$) (Fig. 3).

F3

Iron Concentration in Cell Culture Media

DMEM medium itself contains iron at concentration of 5.48 mg/L (median, 5.7; range, 4.59–6.88 mg/L) (Table 1). After 72 hours of incubation, culture media of colon carcinoma cells and of the fibroblasts contained significantly lower amounts of iron than was initially determined ($p < 0.05$), indicating iron consumption by the growing cell cultures. Significantly lower concentrations ($p < 0.05$) of iron were measured in culture media of Wi38 fibroblasts than in culture media of SW620 colon carcinoma cells after 72 hours, indicating a higher capacity of fibroblasts to utilize iron from the culture media.

T1

Consumption of iron from culture medium by Wi38 fibroblasts was regular, also if the cells were cultured in the presence of FSC iron at the 10^{-5} M or 10^{-4} M concentrations. If the cells were treated with the highest concentration of FSC iron (10^{-3} M), a transient decrease was observed, but the values returned almost to the initial values after 72 hours, indicating the release of iron from the cells.

Similar results were obtained for SW620 colon carcinoma cell cultures. Significantly lower concentrations of iron were measured in culture media of SW620 colon carcinoma cells (Table 1). However, in the case of colon carcinoma cells, the transient decrease of iron values in the culture medium was observed also if the cells were treated with a low concentration of FSC iron. On the other hand, the rise in iron values in the culture medium after a transient decrease was not as prominent as was observed for the fibroblasts.

Table 1. Iron Concentration (Mean Values) in Supernatant of Culture Media of Colon Carcinoma Cells (SW620) and Fibroblast Cells (Wi38) that were Treated with Ferric-Sorbitol-Citrate

		Time of cell culturing (hours)				
Cells	*Iron concentration (M)*	*0*	*3*	*24*	*48*	*72*
SW620	0	5.48	5.56	4.01*	4.60	4.11*
	10^{-5}	5.88	5.00	3.75*	5.26	5.34
	10^{-4}	8.25	3.73*	3.82**	4.89*	4.04*
	10^{-3}	9.73	6.77*	6.03*	6.85*	7.16
Wi38	0	5.48	4.59	4.48	3.05*	2.55*
	10^{-5}	5.88	5.13	4.00	3.01*	2.60**
	10^{-4}	8.25	4.13**	4.79**	4.00**	2.93**
	10^{-3}	9.73	6.83*	4.71**	5.69*	8.09

*$p < 0.05$, when compared to the level of iron concentration in medium at the beginning of the incubation; **$p < 0.01$, when compared to the level of iron concentration in medium at the beginning of the incubation.

The Effect of FSC Iron on Lipid Peroxidation Induction

Results obtained by the analysis of LOOH from human plasma fatty acids under the influence of FSC iron are presented on Table 2. A rise in LOOH content in plasma was observed only after 3 days of incubation in the air atmosphere, even without FCS iron. However, there was no significant difference at any timepoint between plasma samples treated by FSC iron, independent of the iron concentration used and respective controls (for all, $p > 0.05$), suggesting that FSC iron is chemically stable and has no capacity to act as a lipid pro-oxidant.

DISCUSSION

Receptors for transferrin TfR1 on tumor cells are required for the increased DNA synthesis, multiplication, and proliferation of malignant cells.[17] This study showed that TfR1 are equally expressed on nonmalignant human fibroblasts and on malignant colon, laryngeal, and pancreatic carcinoma cells cultured *in vitro*. The expression of TfR1 by colon carcinoma cells and laryngeal carcinoma cells decreased when the cells were incubated in FCS iron-supplemented media. This regulation of TfR1 probably serves as protection from iron overload, which might cause cell damage. On the other hand, iron deprivation seems to prevent cellular progression from G1 to the S-phase of the cell cycle.[11]

Because in the presence of FSC iron SW620 colon carcinoma cells and Hep laryngeal carcinoma cells reduced the expression of TfR1, unlike nonmalignant Wi38 fibroblasts and Mia-PaCa2 pancreatic carcinoma cells, this mechanism might explain a different sensitivity of the cells to the growth-inhibitory effects of FSC iron. Treatment with FSC iron did not change the expression of TfR1 on pancreatic carcinoma cells. Under pathological conditions (e.g., iron overload or serum transferrin deficiency), pancreatic cells store excessive iron,[18] which could explain equal levels of TfR1 expression on pancreatic carcinoma cells in the plain culture medium and in the medium containing FSC iron.

The expression of TfR1 on colon carcinoma cells decreased after incubation with FSC iron for 3 hours, depending on the iron concentration, whereas incubation for 24 hours enhanced that effect. Thus, the expression of TfR1 depended on the FSC iron concentration and on the duration of exposure. The decrease of TfR1-positive malignant cells after longer incubation with FSC iron could be the result of an antitumor mechanism of iron. As we previously described, FSC iron inhibits tumor cells in the G1 and early S-phase; hence, the cells cannot proceed to the S-phase in which the receptor density is high.[19,20]

The differences in TfR1 expression could further be reflected by the expression of ferritin that stores intracellular iron. Namely, a high percentage of colon carcinoma cells cultured with FSC iron for 3 hours contained ferritin, whereas after 24 hours of incubation in higher concentrations of iron, the percentage of ferritin-positive colon carcinoma cells declined. We suppose that the withdrawal of TfR1—caused by iron supple-

Table 2. The Effect of Ferric-Sorbitol-Citrate (FSC) Iron on Lipid Peroxidation of Lyophilized Human Plasma Determined by the Ferric Thiocyanate Method for Detection of Lipid Hydroperoxides (LOOH)

Reaction time/hour	*FSC iron concentration*	$10^6 \times$ *LOOH/mol dm*$^{-3}$
24	0	2.8 ± 1.8
	10^{-5} M[a]	2.9 ± 0.0
	10^{-3} M[b]	
48	0	3.0 ± 0.3
	10^{-5} M	2.9 ± 0.0
	10^{-3} M	3.1 ± 0.1
72	0	3.9 ± 0.3
	10^{-5} M	4.4 ± 0.5
	10^{-3} M	3.7 ± 0.4

Note. Control plasma LOOH values were before any treatment determined as 2.5 ± 1.0 × 10^6 × LOOH/mol dm^{-3}.
[a]Equal to 0.09 × 10^3 × Fe^{III}/mol dm^{-3}.
[b]Equal to 9.0 × 10^3 × Fe^{III}/mol dm^{-3}.

mentation—induced iron release from cellular ferritin, resulting in iron depletion. Iron depletion would cause cellular decay or at least the inability of cells to proliferate.

FSC iron did not change the expression of TfR1 on nonmalignant, normal human Wi38 fibroblasts, neither for the 3 hours of incubation nor for the 24 hours of incubation. Although a tendency of a slight rise of TfR1 was noticed, this was not significant. On the other hand, incubation with FSC iron increased the expression of ferritin in Wi38 fibroblasts, which is the opposite of the effects on colon carcinoma cells. It is known that in normal cells, a major function of intracellular ferritin is to store and detoxify intracellular iron, thus decreasing the ability of iron to promote the generation of reactive oxygen species,[21] which is the major mechanism of iron toxicity.[22] Recent data support the hypothesis that ferritin has iron-detoxifying functions and that this could be a reason for the absence of iron toxicity in normal (nonmalignant) cells.[4,23] It is obvious that nonmalignant fibroblasts have a very prominent capacity to utilize and store iron from FSC, whereas malignant colon carcinoma cells have shown the opposite. Such differences in iron metabolism could be important in the understanding of mechanisms for low iron toxicity for normal (nonmalignant) cells.

In medium without the addition of FSC, iron colon carcinoma cells utilized iron continuously for 72 hours, whereas nonmalignant fibroblasts did the same, even if the medium contained lower doses of FSC iron (10^{-4} or 10^{-5} M). In a higher FSC iron concentration (10^{-3} M), an increased iron uptake was noticed initially, but this led to the decay of the cells, as we have shown in a previous study.[7] Malignant SW620 colon carcinoma cells were at two orders of magnitude more sensitive to this cytotoxic effect of FSC iron than nonmalignant Wi38 fibroblasts, because they have the very same pattern of reaction to the FSC iron treatment as was observed for colon carcinoma cells, even if treated with 10^{-4} or 10^{-5} M FSC iron, whereas fibroblasts exerted cytotoxic effects of FSC iron only if treated with 10^{-3} M iron FSC.[7–9]

It is not likely that cytotoxicity of FSC iron could result from potential pro-oxidative effects of iron. Namely, FSC iron did not induce the lipid peroxidation of human plasma. This is in agreement with our previous findings.[19] However, it should be mentioned that the effects of Fe^{III} on lipid peroxidation are still unclear *in vivo*. Recent investigations suggest the importance of both Fe^{III} and Fe^{II} release from storage molecules as cofactors of lipid peroxidation. The oxidation state of iron is relevant for lipid peroxidation.[24] This process depends on the Fe^{II}/Fe^{III} ratio during the reaction. Inefficiency of FSC iron in our model of lipid peroxidation could be attributed to sorbitol, an isomer of mannitol, which is a very efficient $^{\cdot}OH$ scavenger, as well as on the potent antioxidant capacity of plasma antioxidants.[25] However, as we described, that FSC iron treatment could induce a moderate rise in the intracellular level of lipid peroxidation product 4-hydroxynonenal (HNE), but not severe HNE-mediated oxidative stress, the possibility that altered iron metabolism upon FSC iron treatment could eventually result in intracellular lipid peroxidation should be considered.[19] If so, even a moderate rise in HNE, known also as a second messenger of free radicals and a growth-regulating signaling molecule, could affect proliferation, differentiation, and apoptosis of malignant cells.[26–28] This is especially relevant for colon carcinoma cells, as it is known that colon carcinogenesis is supported by the mutagenic and growth-regulating capacity of HNE and the related product of lipid peroxidation.[29–31]

Recently, we have shown that the majority of patients with colorectal carcinoma suffer from anemia, whereas the density of TfR1 receptors in colorectal carcinomas was increased, especially in the early stages of the disease.[32] Because FSC iron used in this study inhibited the proliferation of colon carcinoma cells *in vitro* and reduced the expression of TfR1 on these cells, and whereas it is used in daily medical practice as an antianemic drug, we suppose that it could have effects as a cancer biotherapeutic drug in patients with colorectal carcinoma, especially in those with Dukes stage A and B. To test this hypothesis, we plan to study the effect of FSC on TfR1 expression on colorectal carcinoma and colon mucus samples *in vitro*, as well as a further molecular mechanism of anticancer effects of FSC iron.

CONCLUSIONS

Our study indicates that the inhibitory effects of ferric-sorbitol-citrate iron on cultured carcinoma cells could be attributed to an initial high utilization of iron, resulting in a negative signal for the expression of TfR1 in tumor cells. Tumor

cells accumulate lower amounts of ferritin than normal fibroblasts and are, therefore, less able to survive consequences of oxidative stress damage caused by iron overload.

ACKNOWLEDGMENTS

The study was supported by the Croatian Ministry of Science Education and Sports. The authors wish to express their gratitude to Dr. Milivoj Boranic for fruitful discussion and language revision of the manuscript, and to Dr. Marko Kralik for support in the statistic analysis of results.

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