Effect of Hydroxyurea on Cellular Iron Metabolism in Human Leukemic CCRF-CEM Cells: Changes in Iron Uptake and the Regulation of Transferrin Receptor and Ferritin Gene Expression following Inhibition of DNA Synthesis

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ABSTRACT

Hydroxyurea inhibits cellular proliferation through action on ribonucleotide reductase, an iron-dependent enzyme responsible for the synthesis of deoxyribonucleotides. Whereas previous investigations have examined the interaction of hydroxyurea with this enzyme, the action of hydroxyurea on other aspects of iron metabolism has not been studied in detail. In our study, incubation of CCRF-CEM cells with hydroxyurea resulted in an inhibition of ribonucleotide reductase activity/DNA synthesis within 4 h and produced a parallel decrease in the uptake of iron by cells. In contrast, iron uptake by hydroxyurea-resistant CCRF-CEM cells was not inhibited by hydroxyurea. After 6 h, hydroxyurea produced an increase in the activity of the iron-regulatory protein, a cytoplasmic mRNA-binding protein responsible for regulating the translation of transferrin receptor and ferritin mRNAs. After 24 h, hydroxyurea-treated cells displayed a 1.5-fold increase in transferrin receptor mRNA and protein and a significant decrease in ferritin levels. The hydroxyurea-induced increase in transferrin receptor was abrogated by transferrin-iron. In contrast to hydroxyurea, inhibition of DNA synthesis by 1-ß-D-arabinofuranosylcytosine produced a decrease in transferrin receptor expression. Our studies suggest that iron uptake by CCRF-CEM cells is closely linked to ribonucleotide reductase activity rather than to transferrin receptor number. Inhibition of ribonucleotide reductase/DNA synthesis by hydroxyurea results in a decrease in iron uptake by cells and an increase in the activity of the iron-regulatory protein, which, in turn, is responsible for the hydroxyurea-induced increase in transferrin receptor and decrease in ferritin synthesis.

INTRODUCTION

Hydroxyurea is a cancer chemotherapeutic drug that has been in use for many years. It is used extensively for the treatment of myeloproliferative disorders (chronic myeloid leukemia, polycythemia vera, and essential thrombocytemia), and it also has clinical activity in head and neck cancer, brain tumors, non-small cell lung cancer, and cancer of the uterine cervix (1). The primary target of action of hydroxyurea is the enzyme ribonucleotide reductase. This enzyme is responsible for the reduction of ribonucleotides to deoxyribonucleotides, a rate-limiting step in DNA synthesis (2). Mammalian ribonucleotide reductase consists of a large (R1) and a small (R2) dimeric subunit. The R1 subunit contains substrate- and effector-binding sites, whereas the R2 subunit contains μ-oxo-bridged iron atoms and a tyrosyl free radical that gives a characteristic signal on ESR spectroscopy (2, 3). Enzyme activity and the tyrosyl radical ESR signal}

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1 Supported by USPHS Grant R01 CA41740, the Ralph and Marion Falk Foundation Trust Grant awarded to the Cancer Center of the Medical College of Wisconsin, and by research funds from the Sampson family. 2 To whom requests for reprints should be addressed, at Division of Hematology-Oncology, Medical College of Wisconsin, 8700 West Wisconsin Avenue, Milwaukee, WI 53226. 3 The abbreviations used are: ESR, electron spin resonance; IRP, iron-regulatory protein; NO, nitric oxide.

increase as cells enter S phase and are dependent on the presence of iron (4). Hydroxyurea inhibits ribonucleotide reductase by inactivating the tyrosyl radical of the R2 subunit, whereas iron chelators, by limiting the availability of iron, also inhibit the R2 subunit and consequently, ribonucleotide reductase enzyme activity (3, 5–9). The inhibition of R2 by hydroxyurea can be partially reversed by ferrous iron, thus indicating involvement of nonheme iron in the action of hydroxyurea on ribonucleotide reductase (6, 10).

Whereas there has been significant investigation into the interaction of iron and hydroxyurea at the level of ribonucleotide reductase activity, limited information exists regarding the action of hydroxyurea on other aspects of iron metabolism. Evidence that the action of hydroxyurea on iron metabolism may extend beyond ribonucleotide reductase has been provided by studies on cell lines that are resistant to the growth-inhibitory effects of hydroxyurea. These hydroxyurea-resistant cells overproduce ribonucleotide reductase and also have an increase in the expression of ferritin, a large molecular weight iron storage protein (11). Earlier studies reported that CCRF-CEM cells exposed to hydroxyurea had an increase in transferrin receptors that mediate the uptake of transferrin-iron by cells (12). However, the basis for the hydroxyurea-induced increase in transferrin receptors was not fully explained.

In the present investigation, we have attempted to advance our understanding of the action of hydroxyurea on iron metabolism by examining the effects of this drug on iron transport into cells and the molecular regulation of transferrin receptor and ferritin gene expression. We show that the inhibition of DNA synthesis by hydroxyurea results in a decrease in iron uptake by cells and a depletion of intracellular iron, leading to an increase in transferrin receptors and a decrease in ferritin levels. This hydroxyurea-induced change in transferrin receptor and ferritin expression is due to an increase in the activity of a cytoplasmic iron-responsive RNA-binding protein responsible for regulating the translation of transferrin receptor and ferritin mRNAs (13, 14).

MATERIALS AND METHODS

Human transferrin and BSA were purchased from Sigma Chemical Co. (St. Louis, MO). Hydroxyurea and 1-ß-D-arabinofuranosylcytosine were obtained from Calbiochem (La Jolla, CA) and Upjohn Co. (Kalamazoo, MI), respectively. [32P]GTP, [32P]CTP, [3H]thymidine, 59FeCl₃, and 125I-sodium were purchased from Amersham (Arlington Heights, IL). 59Fe-transferrin was prepared according to the method of Bates and Schlabach (15), and 59Fe-transferrin was iodinated using the Chloramine T method (16).

Tissue Culture and Incubation Conditions. Human T lymphoblastic leukemia CCRF-CEM cells were obtained from American Type Culture Collection (Rockville, MD) and were incubated in an atmosphere of 6% CO₂ in RPMI 1640 supplemented with 10% FCS. A subline of CCRF-CEM cells relatively resistant to the growth-inhibitory effects of hydroxyurea was developed by incubating cells in a step-wise fashion with increasing concentrations of hydroxyurea. Hydroxyurea-resistant cells were maintained in medium containing 90 μM hydroxyurea and were approximately 7-fold more resistant to growth inhibition by hydroxyurea than were wild-type cells. The IC₅₀ for
wild-type and hydroxyurea-resistant cells were 80 and 556 μm, respectively, after a 72-h incubation with hydroxyurea.

[^3H]Thymidine Uptake. Cells were plated in 96-well plates with increasing concentrations of hydroxyurea (0–500 μm), and[^3H]thymidine (1 μCi/well) was added to wells at the onset of incubation. Cells were harvested onto a glass fiber filter using a Mini-Mash harvester (M. A. Bioproducts, Walkersville, MD), and discs corresponding to individual wells were cut out of the filter. The radioactivity in each disc was counted in a LKB scintillation counter.

ESR Spectroscopy Studies. Studies of the tyrosyl free radical of the R2 subunit of ribonucleotide reductase were performed on intact CCRF-CEM cells that had been incubated for 4 h in culture medium with or without 250 μM hydroxyurea. X-band ESR spectra were obtained using a standard Century series Varian E-100 spectrometer operating at X-band (9–9.5 GHz) and using 100-KHz field modulation. Direct ESR measurements were carried out on frozen samples of 5 × 10^6 cells (0.5 ml) maintained at −196°C in quartz finger dewars as described previously (17). ESR spectra were recorded multiple times and were averaged by computer.

[^59Fe]Uptake Studies. Cells (2 × 10^7/well) were plated in 1-ml multiwell plates in medium with or without hydroxyurea. 59Fe-transferrin (15,000 cpm 59Fe, 3.7 μg transferrin/ml) was added to the culture medium at the start of the incubation. Cells were harvested from the wells after 3, 6, and 24 h of incubation at 37°C or 4°C, washed by centrifugation, and the 59Fe radioactivity in the cell pellet was counted in an LKB Compugamma γ counter.

^125I-labeled Transferrin Binding Studies. Transferrin receptor density on cells was determined using a previously described ^125I-labeled transferrin binding assay (18). Binding studies were performed on intact cells at 4°C, and maximal transferrin binding was determined according to the method of Scatchard (19). For experiments examining the effect of iron on hydroxyurea-induced changes in transferrin receptor expression, cells were incubated with 250 μM hydroxyurea with or without 50 μg/ml of transferrin-iron for 24 h. Before the binding assay, cells were washed with PBS and preincubated in 15 ml of PBS-BSA at 37°C for 30 min to deplete cells of transferrin and make available all the transferrin-binding sites for ^125I-labeled transferrin binding (18).

RNA Isolation and Northern Blotting. Total cellular RNA was isolated by a modification of the method of Chomczynski and Sacchi (20) using RNazol (Tel-Test, Inc., Friendswood, TX) according to the manufacturer’s recommendations. RNA samples (20 μg) were electrophoresed on a 1% agarose gel containing 2.2 M formaldehyde and transferred to Nytran membranes (Schleicher & Schuell, Keene, NH) using capillary-blotting method. Transferrin receptor mRNA was detected by hybridization of the membranes with a 32P-labeled transferrin receptor cDNA (10^6 cpm/ml), as described previously (21). The membranes were exposed to XAR-5 film (Eastman Kodak Co., Rochester, NY) with intensifying screens at −70°C for 24 h. For quantitation, the band intensities were scanned on an AMBIS Optical Imaging System (AMBIS, San Diego, CA).

Protein mRNA-binding Assay. The binding of transferrin receptor and ferritin mRNAs to a regulatory cytoplasmic protein, the IRP, (formerly termed the iron-responsive element-binding protein) was examined by an RNA bandshift assay as described by Leibold and Munro (22). The IRP binds specifically to structural motifs (IREs) on ferritin and transferrin receptor mRNAs (13, 14). After incubation with or without hydroxyurea, cells were washed by centrifugation with PBS and were lysed by resuspending the cell pellet in 20 mM HEPES pH 7.5 buffer containing 5% glycerol, 0.5 mM EDTA, 25 mM KCl, 1 mM DTT, 1% NP40, and 1 mM phenylmethylsulfonyl fluoride. Cellular debris was removed by centrifugation of the lysate at 30,000 × g for 30 min, and the supernatant was analyzed for IRP activity (protein-RNA binding). 32P-labeled IRE-RNA for the RNA band shift assay was prepared using, as a template, a 1000-bp rat ferritin L pseudogene that contains the conserved IRE sequence (generously provided by Dr. Leibold; Ref. 22). The plasmid (p66-L gene) containing this 1000-bp insert was linearized with enzyme SmaI (GIBCO-BRL, Gaithersburg, MD) and was used for in vitro transcription of the IRE-mRNA. Transcription was carried out with Sp6 RNA polymerase using a commercially available transcription system from Promega (Madison, WI). The 32P-labeled RNA was purified on a 5% urea polyacrylamide gel. For the gel retardation assay, 40 μg of cytoplasmic extract from cells were incubated with 100,000 cpm of 32P-labeled IRE-RNA in 10 mM HEPES (pH 7.6)-3 mM MgCl2-40 mM KCl-5% glycerol-1 mM DTT. After a 30-min incubation of the reaction mixture at room temperature, 1 unit RNase T1 was added, and the incubation was continued for an additional 10 min. Five mg/ml heparin were then added to the reaction, and the incubation was continued for another 10 min at room temperature. The mixture was resolved on a 5% non-denaturing polyacrylamide gel, and autoradiography of the dried gel was performed. Band intensities of the autoradiograph were determined by scanning on an AMBIS Optical Imaging System.

Measurement of Cellular Ferritin. Ferritin levels in cells were measured before and after a 24-h incubation with hydroxyurea. For the assay, cells were washed with PBS, disrupted by sonication, and cellular debris was removed by centrifugation (30,000 × g for 30 min). The cytoplasmatic fraction (supernatant) was assayed for protein content and for total ferritin using an assay (Quantimune ferritin assay) from Bio-Rad (Richmond, CA). The ferritin content was expressed as ng/mg of protein.

RESULTS

Hydroxyurea is known to inhibit DNA synthesis by action on the iron-dependent R2 subunit of ribonucleotide reductase (5). Consistent with this effect on DNA synthesis, CCRF-CEM cells incubated with increasing concentrations of hydroxyurea displayed a dose-dependent decrease in the incorporation of [^3H]thymidine during a 4-h incubation (Fig. 1A). To confirm that the hydroxyurea-induced decrease in [^3H]thymidine uptake was due to an inhibition of ribonucleotide reductase, the ESR signal of the R2 subunit of this enzyme was...
HYDROXYUREA AND IRON METABOLISM

At later time points, $^{59}$Fe uptake by control cells continued to increase whereas $^{59}$Fe uptake by hydroxyurea-treated cells increased only slightly. Hydroxyurea is known to possess metal-binding properties (24), and we considered whether its inhibitory effect on iron uptake was the result of chelation of iron after its release from transferrin. To address this possibility, the effect of hydroxyurea on iron uptake was examined using hydroxyurea-resistant cells. As shown in Fig. 2B, hydroxyurea, on a mole-to-mole basis, produced a far greater inhibition of iron uptake in wild-type CCRF-CEM cells than in hydroxyurea-resistant cells. This indicates that the decrease in iron uptake by hydroxyurea-resistant wild-type CCRF-CEM cells was primarily due to the inhibitory action of hydroxyurea on DNA synthesis rather than to chelation of iron by the drug.

Cells were analyzed for the expression of transferrin receptors at various periods after their incubation with hydroxyurea. No significant differences between control and hydroxyurea-treated cells were noted after 6 h of incubation; however, after 18–24 h of exposure of cells to 250 $\mu$M hydroxyurea, transferrin receptor density was increased 1.3–1.6 fold (range of 6 experiments). As shown in the Scatchard analysis plot in Fig. 3A, hydroxyurea-treated cells displayed an increase in cell surface transferrin receptors without a significant change in receptor affinity for transferrin ($K_d = 10.1 \times 10^{-10}$ M versus $8.97 \times 10^{-10}$ M for control versus hydroxyurea-treated cells, respectively). In parallel with the increase in transferrin receptor protein, transferrin receptor mRNA also increased by approximately 1.5-fold after exposure of cells to hydroxyurea (Fig. 3B).

Hydroxyurea is known to arrest cells in G$_1$-S; we therefore considered whether the hydroxyurea-induced increase in transferrin receptors was related to an effect of the drug on the cell cycle. Transferrin receptors were measured on cells incubated with 1-ß-D-arabinofuranosylcytosine, a drug that also produces a G$_1$-S arrest. In contrast to the increase in transferrin binding seen with hydroxyurea,
The above experiments suggested that the hydroxyurea-induced increase in transferrin receptors was due to changes in cellular iron status. To investigate this possibility, 125I-labeled transferrin binding assays were performed on cells that had been coincubated with transferrin-iron and hydroxyurea. As shown in Table 1, cells incubated with hydroxyurea had an increase in transferrin receptors; however, this effect was abrogated by 50 μg/ml transferrin-iron. It was interesting that although transferrin-iron restored transferrin receptor expression to control levels, it did not reverse the growth-inhibitory effects of hydroxyurea. In the experiment shown in Table 1, control cells had approximately doubled in number after a 24-h incubation, whereas cells exposed to hydroxyurea alone or hydroxyurea plus transferrin-iron remained close to the initial plating density.

<table>
<thead>
<tr>
<th>Additive</th>
<th>125I-labeled transferrin binding (%)</th>
<th>Cell count (%)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>159 ± 8.2</td>
<td>100</td>
</tr>
<tr>
<td>HU</td>
<td>159 ± 8.2</td>
<td>159 ± 8.2</td>
</tr>
<tr>
<td>HU + Tf-Fe</td>
<td>100 ± 11.4</td>
<td>54 ± 9.8</td>
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Table 1. Effect of transferrin-iron on hydroxyurea-induced changes in transferrin receptor expression and cellular proliferation.

* Assays were performed after a 24-h incubation. Data represent mean ± SE of 3 separate experiments.

HU, 250 μM hydroxyurea; Tf-Fe, 50 μg/ml transferrin-iron.

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**Fig. 4.** Effect of hydroxyurea on cellular ferritin content. Ferritin levels were measured in cells before (0 h) and after a 24-h incubation in medium. Control, no additives; HU 100, 100 μM hydroxyurea; HU 250, 250 μM hydroxyurea. Columns, mean; bars, range of 2-3 separate experiments.

**Fig. 5.** RNA band-shift assay of lysates from control and hydroxyurea-treated cells. IRP binding to 32P-labeled mRNA IRE (IRP-binding activity) was determined after incubation of cells for the times specified. Forty μg of cytoplasmic extract from cells were assayed as described in "Materials and Methods." Top, autoradiograph of the band shift assay. Bottom, relative band intensities obtained on densitometry of the autoradiograph. The band intensities for the different time points are expressed relative that of the control at 6 h. HU, hydroxyurea. Two separate experiments produced similar results.

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**Fig. 4** shows the effect of hydroxyurea on cellular ferritin content. Ferritin levels were measured before and after a 24-h incubation in medium. Control, no additives; HU 100, 100 μM hydroxyurea; HU 250, 250 μM hydroxyurea. The figure includes a bar graph and a table summarizing the results. Overall, hydroxyurea led to a decrease in ferritin levels, which was more pronounced at 24 h. The table provides more detailed data, showing that the decrease in ferritin was significant compared to control conditions.

**Fig. 5** illustrates a RNA band-shift assay of lysates from control and hydroxyurea-treated cells. The top panel shows the autoradiograph of the band shift assay, while the bottom panel displays the relative band intensities obtained on densitometry of the autoradiograph. The data indicate a progressive increase in IRP activity from 6 to 24 h in hydroxyurea-treated cells. The table below the figure summarizes these findings, with control conditions showing lower band intensities compared to hydroxyurea-treated cells.

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**Table 1 Effect of transferrin-iron on hydroxyurea-induced changes in transferrin receptor expression and cellular proliferation**

<table>
<thead>
<tr>
<th>Additive</th>
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<td>HU + Tf-Fe</td>
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<td>54 ± 9.8</td>
</tr>
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</table>

* Assays were performed after a 24-h incubation. Data represent mean ± SE of 3 separate experiments.

HU, 250 μM hydroxyurea; Tf-Fe, 50 μg/ml transferrin-iron.
DISCUSSION

Our study shows that after the inhibition of ribonucleotide reductase/DNA synthesis by hydroxyurea, the following sequence of events in iron metabolism occur: (a) by 3–4 h there is a decrease in iron uptake by cells; (b) by 6 h there is a progressive increase in the binding of the IRP to transferrin receptor and ferritin mRNAs; and (c) by 18–20 h there is an increase in steady-state transferrin receptor mRNA and protein and a decrease in ferritin. The ability of excess iron to reverse the increase in transferrin receptor expression confirms that the diminution in cellular iron is the key event responsible for these hydroxyurea-induced changes in transferrin receptor and ferritin levels.

The uptake of iron (as transferrin-iron) by cells is mediated by specific cell surface receptors for transferrin (28). The expression of transferrin receptors increases during S phase, and this is felt to reflect the need for iron to support cellular proliferation (29–33). It would be anticipated, therefore, that the inhibition of DNA synthesis by hydroxyurea would produce a decrease in transferrin receptors. Indeed, inhibition of DNA synthesis by 1-ß-D-arabinofuranosylcytosine did lead to a decrease in cellular transferrin receptors. In contrast, however, exposure of CCRF-CEM cells to hydroxyurea for 18–24 h resulted in an increase in transferrin receptor mRNA and protein. These findings are consistent with a prior study by Hedley et al. (12) that reported that hydroxyurea produced an increase in immunofluorescent cell surface transferrin receptors and an arrest of CCRF-CEM cells in S phase. Our studies now provide an explanation for this earlier observation in the context of our current understanding of the molecular regulation of transferrin receptor expression. We show that the up-regulation of transferrin receptor levels in hydroxyurea-treated cells is due to an increase in the activity of the IRP, a cytoplasmic protein that plays a central role in the iron-dependent regulation of transferrin and ferritin mRNA translation. Thus, incubation of cells with hydroxyurea changes the IRP from a low to a high affinity form, which then binds to transferrin receptor and ferritin receptor mRNAs. The net result is an increase in transferrin receptor mRNA translation and a repression of ferritin mRNA translation.

How does hydroxyurea produce this increase in IRP activity beyond that seen in control cells? Because the activity of the IRP is altered by changes in cellular iron status, the likely explanation is that the hydroxyurea-induced decrease in cellular iron uptake reduces cellular iron to below a critical level and that this leads to the conversion of the IRP to a high affinity mRNA-binding form. However, an additional mechanism of action of hydroxyurea could theoretically be involved. The continuous increase in IRP activity and marked repression of ferritin mRNA translation by hydroxyurea suggests that hydroxyurea may, by virtue of its iron-binding property, act directly on the IRP and alter its affinity state. Because no information exists regarding interaction between hydroxyurea and the IRP, further studies are warranted to determine whether such an interaction does exist and whether it contributes to changes in transferrin receptor and ferritin expression. Furthermore, the possibility that hydroxyurea may directly increase IRP activity by removing its iron raises questions regarding potential interactions of hydroxyurea with other iron-containing molecules. In particular, the iron-sulfur cluster of mitochondrial aconitase (and other iron-containing sites in mitochondria; Ref. 34) may also prove to be targets for hydroxyurea. Earlier studies demonstrated that the inhibition of deoxyribonucleotide synthesis by hydroxyurea in intact HeLa cells could only be partially reversed by exogenous deoxyribonucleotides (35), thus supporting the idea that the site of action of hydroxyurea may extend beyond ribonucleotide reductase.

There appear to be intriguing parallels between the actions of hydroxyurea and NO on ribonucleotide reductase and IRP activity. NO readily reacts with iron, iron-sulfur centers, and thiols (36) and inhibits ribonucleotide reductase by destroying the tyrosyl radical of its R2 subunit (37, 38). Recent studies utilizing J774.A1 murine macrophage cell lines have shown that NO increases IRP activity (39, 40) and that cytokine-inducible NO synthase mRNA transcription (and hence enzyme activity) is increased by iron deprivation and decreased by iron excess (41). Of significance is that hydroxyurea resembles Nω-hydroxy-L-arginine, an intermediate in NO production by macrophage NO synthase, and that hydroxyurea can generate NO in vitro in the presence of H2O2 and CuSO4 (38). Whereas a role for NO in the hydroxyurea-induced increase in IRP activity is speculative at present, it is conceivable that such a mechanism could be at play in intact cells.

Our investigation underscores the important relationship of iron uptake to intracellular iron and ribonucleotide reductase activity. In addition to showing that IRP activity in hydroxyurea-treated cells was greater than control cells at all times, Fig. 5 also shows that IRP activity in control cells increased progressively from 6 to 24 h and then decreased significantly by 48 h. This finding in control cells is consistent with other studies in different cell types (42, 43) and explains the mechanism responsible for the increase in transferrin receptors during cellular proliferation. Collectively, several studies have suggested that during proliferation intracellular iron is rapidly utilized (for cellular functions), resulting in depletion of a putative, regulatory iron "pool" within the cell. This, in turn, results in an increase in IRP activity and an increase in transferrin receptor synthesis (42, 43). Our present work extends these earlier studies and suggests that the regulatory intracellular iron pool responsible for the increase in IRP activity during proliferation is the same iron pool that becomes depleted when ribonucleotide reductase activity and iron uptake are inhibited by hydroxyurea. Iron transport into this pool is the key factor in maintaining cellular proliferation, whereas changes in transferrin receptor expression per se are dictated by changes in this regulatory iron pool and IRP activity. This concept is further supported by the observation that wild-type CCRF-CEM cells display a marked decrease in iron uptake when ribonucleotide reductase is inhibited by hydroxyurea, whereas hydroxyurea-resistant cells do not. Hence, iron uptake by cells appears to be closely linked to DNA synthesis, and to ribonucleotide reductase in particular, because this enzyme is iron dependent and is rate limiting for DNA synthesis. Conversely, when ribonucleotide reductase activity is inhibited, iron uptake by cells rapidly diminishes, regardless of the number of surface transferrin receptors present on cells at the time.

Because the inhibition of iron uptake after incubation of cells with hydroxyurea does not appear to be due to a direct effect of hydroxyurea on membrane iron transport per se, it is likely that some form of feedback mechanism exists between DNA synthesis and iron transport into cells, and that this mechanism regulates iron uptake in response to ribonucleotide reductase activity. Further investigation of this process may advance our understanding of the utilization of iron by ribonucleotide reductase and may also yield novel insights into the action of hydroxyurea on cells. A better understanding of the interaction of hydroxyurea with iron-dependent processes may allow one to develop effective clinical therapeutic strategies to enhance the antineoplastic activity of hydroxyurea by combining it with other agents that perturb cellular iron metabolism such as antitransferrin receptor antibodies, iron chelators, and gallium nitrate (9, 17, 44, 45).

Note Added In Proof

A second IRP (IRP2) has recently been characterized from human tissues (46). Human IRP1-IRE and IRP2-IRE complexes comigrate in nondenaturing polyacrylamide gels in the RNA band-shift assay. It remains to be determined...
whether the hydroxyurea-induced increase in IRP activity represents an in-
crease in the activity of IRP1, IRP2, or both.

ACKNOWLEDGMENTS

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