Inhibition of Leukemic HL60 Cell Growth by Transferrin-Gallium: Effects on Ribonucleotide Reductase and Demonstration of Drug Synergy With Hydroxyurea

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Cellular requirements for iron during DNA synthesis are related to the increased activity of the iron-containing M2 subunit of ribonucleotide reductase, the enzyme responsible for the reduction of ribonucleotides to deoxyribonucleotides. We have previously shown that transferrin-gallium (Tf-Ga) inhibits cellular iron incorporation. In the present study, Tf-Ga-induced inhibition of HL60 cell growth and upregulation of Tf receptor density was reversed with hemin. Cells exposed to 2 μmol/L Tf-Ga for six hours or longer displayed a diminution in the electron spin resonance (ESR) spectroscopy signal of the tyrosyl radical of the M2 subunit of ribonucleotide reductase. The effect of Tf-Ga on the ESR signal was reversed by hemin.

Recent clinical trials have found the investigational drug gallium nitrate (NSC-15200) to be effective in the treatment of certain malignancies as well as in the treatment of cancer-associated hypercalcemia. Information regarding the mechanism of action of gallium is, however, largely incomplete. Several studies have demonstrated that gallium avidly binds to transferrin (Tf) and lactoferrin and can be incorporated into ferritin. Studies by Larson et al have suggested that the uptake of 67Ga and 59Fe by EMT-6 sarcoma cells is mediated by the same receptor, ie, the Tf receptor. We have recently shown that the Tf-mediated uptake of 67Ga by human promyelocytic leukemic HL60 cells correlates closely with the number of cell surface Tf receptors and that this uptake can be blocked by an anti-Tf receptor monoclonal antibody that inhibits receptor internalization. Furthermore, it has been shown that 67Ga injected intravenously into rabbits binds exclusively to Tf and that the uptake of 67Ga by transplanted melanoma cells in vivo can be blocked by an anti-Tf receptor monoclonal antibody. In this respect therefore, gallium appears to share certain common properties with iron.

Although the mechanism of cytotoxicity of gallium is incompletely understood, our previous studies show that Tf-Ga inhibits iron uptake by HL60 cells and that the growth-inhibitory effects of Tf-Ga can be reversed by iron-Tf but not by other Tf-metals. We have also demonstrated that Tf-Ga can inhibit hemoglobin production by Friend erythroleukemia cells through a similar mechanism. These studies suggest that the cytotoxicity of Tf-Ga is related, at least in part, to inhibition of cellular iron uptake.

The importance of iron in cellular proliferation is well recognized. Iron is transported bound to Tf and is incorporated into cells by receptor-mediated endocytosis of Tf-iron. It is felt that the expression of Tf receptors by rapidly proliferating nonerythroid cells in vitro and in vivo reflects iron requirements for cell growth. Although iron is required for various cellular functions, it is of critical importance for the activity of the enzyme ribonucleotide reductase. This enzyme is responsible for the conversion of ribonucleotides to deoxyribonucleotides, an early and possibly rate-limiting step in DNA synthesis. Mammalian ribonucleotide reductase consists of two nonidentical protein subunits termed M1 and M2. The M2 subunit contains nonheme iron and a tyrosyl free radical that gives a characteristic signal on electron spin resonance (ESR) spectroscopy. The magnitude of the ESR signal produced by the M2 tyrosyl free radical correlates with the activity of the enzyme. The stability of the tyrosyl radical of the M2 subunit is closely linked to the presence of iron in the enzyme, and removal of this iron by chelators such as deferoxamine destroys the radical. The activity of the tyrosyl radical is increased three- to sevenfold when synchronized mouse mammary tumor cells pass from the G1 to the S phase of the cell cycle. Hence, iron requirements for cellular proliferation appear to be directly related to the increased activity of the M2 subunit of ribonucleotide reductase during DNA synthesis.

Since Tf-Ga inhibits iron incorporation into HL60 cells, we hypothesized that growth-inhibitory concentrations of this Tf-metal should be capable of decreasing the activity of ribonucleotide reductase by limiting iron availability to this enzyme. Additionally, since hydroxyurea is known to inhibit DNA synthesis by inhibiting the activity of the M2 subunit of ribonucleotide reductase, we further hypothesized that...
GA inhibits ribonucleotide reductase

TF-Ga should be capable of potentiating the inhibitory effects of hydroxyurea on cellular proliferation.

The studies reported here are a continuation of our earlier investigations into the mechanism of TF-Ga cytotoxicity and the interaction of this TF-metal with cellular iron metabolism. We show that TF-Ga significantly inhibits the ESR signal of the tyrosyl radical of the M2 subunit of ribonucleotide reductase and the synthesis of deoxyribonucleotides. The effects of TF-Ga can be reversed by iron provided to cells as hemin. As a result of its action on ribonucleotide reductase, TF-Ga can act with hydroxyurea in a synergistic fashion to inhibit the proliferation of HL60 cells.

METHODS

Materials. Human TF, ferric chloride, and hemin were purchased from Sigma Chemical Co (St Louis). Gallium nitrate was obtained from Alfa Products (Danvers, MA). Hydroxyurea was obtained from Calbiochem-Behring Corp (San Diego). 125I-sodium iodide and 99FeCl3 were obtained from New England Nuclear (Boston). Saturation of apotransferrin (apo-Tf) with 99Fe and iodination of 99Fe-Tf were performed as previously described.23 TF-Ga was prepared as previously described.1 Briefly, 3 mol of gallium (as gallium nitrate) was added to each mole of apo-Tf dissolved in a 20-mmol/L acetic acid, 150 mmol/L NaCl, pH 3.5, buffer, and the pH of this solution was raised in gradual increments to 7.4 with 1 mol/L NaHCO3. Saturation of Tf by Ga (1 mol of Tf binding to 2 mol of Ga) was confirmed spectrophotometrically by using a DU 40 Beckman spectrophotometer (Beckman Instruments, Inc, Fullerton, CA) and by measuring the change in absorbance (which occurs with saturation of both the metal binding sites of Tf) at wavelength 242 nm.3 All concentrations of TF-Ga used in these studies were expressed as micromoles per liter of protein. Hemin was dissolved in 0.2 mol/L NaHCO3/NaHCO3 buffer, pH 10.7, and maintained as a 1 mmol/L of 100x stock solution. The addition of hemin to media (as described later) did not alter the pH of the media.

Cells and cell growth studies. Human promyelocytic leukemia HL60 cells were maintained in stock flasks in RPMI 1640 media containing 10% fetal calf serum with 200 U/mL of penicillin and 0.2 mg/mL of streptomycin (regular media). The cells were incubated in an atmosphere of 7% CO2 at 37°C and were allowed to grow to confluency before their use in cell growth studies. Stock cells were washed free of regular media and then plated in serum-free RPMI 1640 media containing 5 μg/mL of insulin and 0.125 μmol/L of apo-Tf (defined media). All cell growth experiments were performed in defined media by plating cells in 1-mL multiwell plates at an initial plating density of 5 x 104 cells/mL. TF-Ga, hydroxyurea, and hemin were prepared as 100 x stock solutions, and different concentrations of these agents were added either singly or in combination to the incubation media at time 0. Cells were incubated for 72 hours at 37°C in a CO2 incubator, and cell counts were then performed in triplicate by using a hemocytometer. Cell viability was determined by trypan blue exclusion.

To test whether the combined effects of TF-Ga and hydroxyurea on cellular proliferation were antagonistic, additive, or synergistic, HL60 cells (5 x 104 cells/mL) were incubated for 72 hours with increasing concentrations of a combination of TF-Ga and hydroxyurea at a fixed molar ratio of 1:100. The effects of this combination on cell growth were analyzed according to the method of Chou and Talalay22 with a dose-effect analysis computer program (by Joseph Chou and Ting-Chao Chou, published by Elsevier-BIOSOFT, Cambridge, UK).

Cell cycle analysis. Cell cycle analysis of HL60 cells were performed as previously described by using propidium iodide for DNA staining.23 Cells were incubated for 24 hours with or without 2 μmol/L of TF-Ga and were analyzed by using a Coulter EPICS V flow cytometer (Coulter Electronics, Hialeah, FL).

125I-Tf binding studies. TF receptor density on cells exposed to TF-Ga or TF-Ga plus hemin was measured by using 125I-Tf binding to intact cells as previously described.21 Before the 125I-Tf binding studies, cells were washed twice with phosphate-buffered saline (PBS) and incubated at 37°C for 20 minutes in PBS containing 0.1% bovine serum albumin. This latter preincubation step depletes cells of Tf and makes available all cell surface Tf binding sites for the 125I-Tf binding assay.11 Incubations were carried out at 4°C for three hours. Specific binding was determined as previously described,21 and maximal Tf binding was calculated according to the method of Scatchard.24

ESR spectroscopy studies. Studies of the tryosyl free radical of ribonucleotide reductase were performed on HL60 cells exposed to 2 μmol/L of TF-Ga for 3, 6, 12, 18, or 24 hours. Control cells for each time point were plated in defined media without TF-Ga. X-band ESR spectra were obtained at the National Biomedical ESR Center of the Medical College of Wisconsin by using a standard Century series Varian E-100 spectrometer operating at X-band (9 to 9.5 GHz) with 100 kHz field modulation. Direct ESR measurements were carried out on frozen samples maintained at –196°C in quartz finger dewars. Each sample contained 5 x 106 cells washed free of media to yield a packed cell volume of approximately 0.5 mL. ESR spectra were recorded eight to 16 times and averaged by computer.

14C-Adenosine incorporation in DNA. HL60 cells were plated in 7 mL of defined media (5 x 104 cells/mL) in T25 cm2 flasks. Tf-Ga (2 μmol/L) was added to one set of flasks but was omitted in the control flasks. Following an 18-hour incubation at 37°C, the flasks were pulsed for three hours with 0.5 μCi/mL of 14C-adenosine. Cells were then harvested and washed with ice-cold PBS. The cell pellet was resuspended in 3 mL of 0.4 N perchloric acid and kept on ice for 30 minutes. The cell precipitate was removed by centrifugation (40,000 g for five minutes), washed a second time with 6 mL of 0.4 N perchloric acid containing 0.14 μCi of 14C-thymidine as an internal standard, and the nucleotide pools were analyzed by HPLC using a Whatman Partisil 10 SAX (anion exchange) column that was eluted isocratically with 7 mmol/L Na2HPO4 (pH 4.0) for five minutes, followed by a gradient to 15% of 1 mol/L Na2HPO4 (pH 4.5) in 20 minutes then to 70% of 1 mol/L Na2HPO4 (pH 4.5) over a period of 31 minutes. The flow rate was 1.5 mL/min, and all

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solvents were conditioned by using a silica precolumn. Nucleotides were plated at a density of 5 x 10^5 cells/mL in defined media to authentic standards. Deoxynucleotides were quantitated by comparison of peak heights to standards and by the 260/280 absorbance ratio. Peaks were identified by comparison of retention times to authentic standards and by the 260/280 absorbance ratio. Deoxynucleotides were quantitated by comparison of peak heights to standards.

RESULTS

Effects of Tf-Ga on HL60 cell growth and reversal of cell growth inhibition by hemin. Figure 1 shows the effects of increasing concentrations of Tf-Ga on the proliferation of HL60 cells. The data are consistent with our previously reported studies of Tf-Ga cytotoxicity.\(^\text{11}\) Since we have previously shown that Tf-Ga inhibits cellular iron incorporation and that the growth-inhibitory effects of Tf-Ga can be reversed by Tf-Fe, we sought to further determine whether iron supplied to cells by mechanisms other than the Tf-Fe receptor pathway could also protect cells from Tf-Ga toxicity. As shown in Fig 2, incubation of cells with a growth-inhibitory concentration of Tf-Ga and increasing concentrations of hemin resulted in a progressive restoration of cellular proliferation.

Effects of Tf-Ga and hemin on the expression of cell surface Tf receptors. Incubation of HL60 cells with non-growth-inhibitory and growth-inhibitory concentrations of Tf-Ga leads to an increase in the density of cell surface Tf binding sites.\(^\text{11,21}\) This upregulation of Tf receptors appears to reflect an attempt by cells to overcome the inhibitory effects of Tf-Ga on cellular iron uptake. Table 1 shows that coincubation of cells with Tf-Ga and hemin resulted in a return of cell surface Tf binding sites to levels almost equivalent to the control. These \(^\text{12}\)I-Tf binding studies and the cell growth studies described earlier suggest that hemin is capable of reversing the Tf-Ga–induced state of cellular iron deprivation by directly providing iron to cells. As a result, both cellular proliferation and transferrin receptor density are restored to control levels.

Inhibition of the ESR signal of ribonucleotide reductase by Tf-Ga. Since ribonucleotide reductase is an enzyme known to carry a stable free radical, we examined the activity of the M2 subunit of this enzyme in HL60 cells by using ESR spectroscopy. The ESR spectra obtained from these cells was consistent with the M2 tyrosyl radical ESR spectra described by others.\(^\text{16,18,27}\) HL60 cells were analyzed after incubation with growth-inhibitory concentrations of Tf-Ga for 3, 6, 12, 18, and 24 hours. In Fig 3, the ESR signal from cells exposed to Tf-Ga for different lengths of time is compared with the ESR signal from control cells. After three hours of incubation no significant differences were seen between the two cell populations. However, after six hours, the ESR signal from the control cells had approximately doubled in magnitude (consistent with the onset of DNA synthesis), whereas the ESR signal from Tf-Ga–treated cells failed to show this increase. Subsequent to this, the ESR signal from the control cells remained relatively constant while the magnitude of the ESR signal from cells exposed to Tf-Ga progressively diminished. After 24 hours of incubation, the ESR signal from Tf-Ga–treated cells was approximately 30% that of control cells; however, this signal could be restored to control levels by coincubation of Tf-Ga–treated cells with hemin (Fig 4). During the first 24 hours of incubation, cells exposed to Tf-Ga maintained a cell cycle distribution similar to control cells (Fig 5). Therefore, differences in the ESR signal between control and Tf-Ga–treated cells was not a result of differences in cell cycle distribution between the two cell populations but appeared to be due to an actual decrease in the activity of the M2 subunit of ribonucleotide reductase.

Table 1. Effect of Tf-Ga and Hemin on Cell Surface Tf Receptor Expression

<table>
<thead>
<tr>
<th>Media Additive</th>
<th>Maximal Transferrin Bound, Mean (Range) (ng/10^6 Cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.8 (7.48-8.13)</td>
</tr>
<tr>
<td>Tf-Ga, 1.25 μmol/L</td>
<td>18.7 (16.78-20.66)</td>
</tr>
<tr>
<td>Tf-Ga, 1.25 μmol/L + Hemin, 10 μmol/L</td>
<td>10.8 (10.55-11.06)</td>
</tr>
</tbody>
</table>

Cells were incubated for 48 hours in defined media containing the various additives. Cells were then harvested and assayed for Tf binding sites. Control cells were incubated with 1.25 μmol/L of apo-Tf. Data represent the results of two separate binding studies, each performed in duplicate.
Effect of Tf-Ga on the incorporation of $^{14}$C-adenosine into DNA and on intracellular deoxyribonucleotide pools. The incorporation of $^{14}$C-adenosine into DNA was examined by pulse-labeling cells grown with and without 2 $\mu$mol/L of Tf-Ga. In a separate experiment, the dNTP pools in control and Tf-Ga–treated cells were examined. As shown in Table 2, cells exposed to Tf-Ga incorporated significantly less $^{14}$C-adenosine into DNA and contained smaller deoxyribonucleotide pools than did control cells. The ribonucleotide pools were unchanged (data not shown). These data along with the ESR spectroscopy studies strongly suggest that Tf-Ga inhibits the synthesis of deoxyribonucleotides by inhibiting the activity of ribonucleotide reductase. The magnitude of the decrease in the deoxycytidine triphosphate (dCTP) pool is comparable to that observed following a 24-hour exposure of HL60 to hydroxyurea.

Synergistic effects of Tf-Ga and hydroxyurea on the proliferation of HL60 cells. It is known that hydroxyurea inhibits cellular proliferation through its action on the M2 subunit of ribonucleotide reductase. Hydroxyurea appears to be capable of destroying the tyrosyl radical of this enzyme, and these inhibitory effects of hydroxyurea on ribonucleotide reductase can be potentiated by the presence of iron chelators such as deferoxamine. Since the studies described before suggested that Tf-Ga inhibited ribonucleotide reductase, we sought to determine whether the inhibitory effects of hydroxyurea on cellular proliferation could be enhanced by Tf-Ga. To determine whether the combined effects of hydroxyurea and Tf-Ga on cell growth were synergistic, additive, or antagonistic, studies were performed according to the method described by Chou and Talalay.

Table 3 shows the effects of hydroxyurea and Tf-Ga alone and in combination on the growth of HL60 cells. The data in Table 3 were analyzed by a computer that generated the following ED_{50} (median effect) values: hydroxyurea, 72.41 ± 12.73 $\mu$mol/L; Tf-Ga, 1.1 ± 0.02 $\mu$mol/L; and hydroxyurea plus Tf-Ga (combination), 27.43 ± 1.31 $\mu$mol/L (data represent the mean of three separate experiments ± SD). The combination index, with respect to the fraction inhibited (Fa) by the combination of both agents, was determined.
separately for each of the three experiments, and the values obtained are expressed as the mean and range in Table 4. Since the median effect plots for hydroxyurea, Tf-Ga, and their combination were not parallel to each other (data not shown), it could not be determined whether the effects of the two agents on cell growth were mutually exclusive or mutually nonexclusive. Therefore, the data were analyzed on the basis of both mutually exclusive and mutually nonexclusive assumptions, and as shown in Table 4, the combination index values for the different degrees of \( Fa\) were consistent with synergism (ie, \(< 1\)). Additionally, at higher degrees of \( Fa\), the combination of Tf-Ga and hydroxyurea appeared to be increasingly synergistic.

### DISCUSSION

We have continued our investigation into the mechanism of the cytotoxicity of Tf-Ga and we now show that Tf-Ga inhibits the ESR signal of the iron-containing M2 subunit of ribonucleotide reductase. The magnitude of the ESR signal correlates closely with the activity of the M2 subunit.\(^{16,18}\) However, since the activity of ribonucleotide reductase varies during different phases of the cell cycle, it was important that the control and Tf-Ga–treated cells being analyzed by ESR spectroscopy be comparable. Although differences in the ESR signals from control and Tf-Ga–treated cells were seen after three to six hours of incubation, both cell populations displayed a similar cell cycle distribution, viability, and cell number during the first 24 hours of incubation. Therefore, the decrease in tyrosyl radical ESR signal in Tf-Ga–treated cells was not due to differences in cell cycle but represented an actual diminution in the activity of the M2 subunit of ribonucleotide reductase.

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**Table 2. Effect of Tf-Ga on the Incorporation of \(^{14}\)C-Adenosine Into DNA and on Intracellular Deoxyribonucleotide Pools**

<table>
<thead>
<tr>
<th>dNTP Pool (pmol/(10^6) Cells)†</th>
<th>dATP</th>
<th>dCTP</th>
<th>dTTP</th>
<th>dGTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5,326 ± 344</td>
<td>17.4 ± 1.6</td>
<td>6.8 ± 1.2</td>
<td>23.8 ± 1.8</td>
</tr>
<tr>
<td>Tf-Ga</td>
<td>2,902 ± 631</td>
<td>7.4 ± 1.2</td>
<td>4.3 ± 0.6</td>
<td>17.2 ± 2.3</td>
</tr>
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</table>

**Abbreviations:** dATP, deoxyadenosine triphosphate; dCTP, deoxycytidine triphosphate; dTTP, deoxythymidine triphosphate; dGTP, deoxyguanosine triphosphate.

*Cells were incubated with 2 \(\mu\)mol/L of Tf-Ga or without Tf-Ga (control) for 18 hours followed by a three-hour pulse with \(^{14}\)C-adenosine. Results represent means ± SD of triplicate experiments.

†Deoxynucleotide (dNTP) pools were measured after 20 hours of incubation. Values represent means ± SD of triplicate experiments.

**Table 3. Inhibition of HL60 Cell Growth by Hydroxyurea and Tf-Ga Alone and in Combination**

<table>
<thead>
<tr>
<th>HU ((\mu)mol/L)</th>
<th>Fraction Inhibited ((Fa)) at Tf-Ga ((\mu)mol/L) Concentration of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12.5</td>
<td>.04</td>
</tr>
<tr>
<td>25.0</td>
<td>.44</td>
</tr>
<tr>
<td>100</td>
<td>.66</td>
</tr>
<tr>
<td>125</td>
<td>.70</td>
</tr>
</tbody>
</table>

HL60 cells were plated at 5 × 10^8 cells/mL in defined media containing different concentrations of Tf-Ga or hydroxyurea (HU) or different concentrations of combinations of Tf-Ga and HU at a fixed molar ratio of 1:100. Cell counts were performed after 72 hours of incubation. \(Fa\) by HU, Tf-Ga, or both equals 1 – (cell count/control cell count). Data shown represent the mean of three separate experiments.

**Table 4. Calculated Values for the Combination Index as a Function of \(Fa\) of HL60 Cell Growth by a Mixture of Tf-Ga and Hydroxyurea**

<table>
<thead>
<tr>
<th>(Fa)</th>
<th>Combination Index (Range)</th>
<th>Diagnosis of Combined Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>.19</td>
<td>.80 (.71–.89)</td>
<td>.94 (.91–1.0)</td>
</tr>
<tr>
<td>.29</td>
<td>.72 (.67–.81)</td>
<td>.84 (.77–.95)</td>
</tr>
<tr>
<td>.42</td>
<td>.66 (.58–.74)</td>
<td>.76 (.66–.86)</td>
</tr>
<tr>
<td>.59</td>
<td>.59 (.49–.65)</td>
<td>.68 (.56–.76)</td>
</tr>
<tr>
<td>.70</td>
<td>.56 (.46–.61)</td>
<td>.64 (.51–.71)</td>
</tr>
<tr>
<td>.89</td>
<td>.49 (.40–.57)</td>
<td>.55 (.44–.65)</td>
</tr>
<tr>
<td>.98</td>
<td>.44 (.39–.51)</td>
<td>.48 (.40–.57)</td>
</tr>
<tr>
<td>.99</td>
<td>.42 (.39–.51)</td>
<td>.46 (.41–.55)</td>
</tr>
</tbody>
</table>

The values for \(Fa\) of HL60 cell growth by a mixture of Tf-Ga and hydroxyurea (shown in the far left-hand column) are taken from Table 3. The combination index values for each \(Fa\) represent the mean and range of three separate experiments. Because exclusivity of drug effects could not be determined by the median effect plot for Tf-Ga, hydroxyurea, and their mixture, the data were analyzed for the combination index on the basis of mutually exclusive as well as mutually nonexclusive drug actions. A combination index of \(< 1\) represents drug synergy.\(^{22}\)
Ga inhibits ribonucleotide reductase

We have previously shown that Tf-Ga inhibits cellular iron uptake and that this inhibition leads to an arrest in cellular proliferation, an effect that can be reversed by excess amounts of Tf-Fe. To demonstrate that inhibition of iron availability to cells is the mechanism whereby Tf-Ga inhibits DNA synthesis, mixing experiments were performed by using hmin to reverse the effect of Tf-Ga on cell growth and Tf receptor expression. Iron from hmin can be made available to an intracellular free iron pool that plays a role in the regulation of cellular Tf receptor synthesis. The addition of hmin to the media prevented the Tf-Ga–induced decrease in the ESR signal of ribonucleotide reductase. This protection in a state of intracellular iron deficiency produced by Tf-Ga. This in turn, resulted in a restoration of normal cellular proliferation and a return of Tf receptor density toward baseline levels.

Cells exposed to Tf-Ga incorporated significantly less 14C-adenosine into DNA and contained less deoxyribonucleotide than did control cells. These latter results are in agreement with a recent report in which exposure of cells to Ga resulted in a significant reduction in intracellular deoxyribonucleotide pools. Our study therefore strongly suggests that the inhibition of cellular iron uptake by Tf-Ga leads to a reduction in the amount of intracellular iron available for the activity of the M2 subunit of ribonucleotide reductase. As a result, there is a marked reduction in the synthesis of deoxyribonucleotides and an arrest in cellular proliferation.

Although our studies suggest that Tf-Ga inhibits ribonucleotide reductase activity, we have not excluded the possibility that other iron-dependent processes essential to cell growth and viability are also inhibited. In addition, we have assumed that the effect of Tf-Ga on ribonucleotide reductase is an indirect one, ie, the result of intracellular iron deprivation. Further studies will be needed to determine whether Ga can interfere directly with this enzyme or with other essential steps in cellular iron metabolism.

It has been shown that hydroxyurea blocks the biosynthesis of deoxyribonucleotides by inhibition of ribonucleotide reductase and that the cytotoxicity of hydroxyurea can be enhanced by iron chelators. We therefore hypothesized that Tf-Ga, as a result of its inhibitory effect on cellular iron uptake, should be capable of potentiating the cytotoxicity of hydroxyurea. Our studies show that Tf-Ga and hydroxyurea in combination do indeed act synergistically to inhibit the proliferation of HL60 cells. A plausible mechanism of drug synergy which is based on our data and the literature is that hydroxyurea destroys the tyrosyl radical of the M2 subunit of ribonucleotide reductase without significantly altering the iron site of the M2 subunit while Tf-Ga appears to interfere with the iron-containing component of the M2 subunit. As a result of their actions on different sites of the M2 subunit of the enzyme, these two agents act synergistically to inhibit DNA synthesis.

These combined effects of Ga and hydroxyurea on cellular proliferation are of clinical significance since both drugs are currently being used to treat various malignancies. Because the drugs are capable of exerting synergistic activity, the use of lower doses of each drug in combination may result in a reduction in individual drug toxicities without a loss of significant antineoplastic activity. Further studies evaluating the therapeutic potential and toxicity of combinations of these two agents in in vivo tumor models thus appear to be warranted.

Our studies have further elucidated the mechanism of cytotoxicity of Tf-Ga and provide a basis for the in vivo evaluation of Ga in combination with hydroxyurea for the treatment of malignancies in which tumor cells may display significant numbers of Tf receptors (eg, lymphomas and polycythemia vera). Further investigations into the cellular handling of Ga may reveal additional important interactions between Ga and iron or Ga and other macromolecules.

REFERENCES

Inhibition of leukemic HL60 cell growth by transferrin-gallium: effects on ribonucleotide reductase and demonstration of drug synergy with hydroxyurea [see comments]

CR Chitambar, WG Matthaeus, WE Antholine, K Graff and WJ O'Brien