Characterization of a Hydroxyurea-resistant Human KB Cell Line with Supersensitivity to 6-Thioguanine

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Abstract

Hydroxyurea (HU) is currently used in the clinic for the treatment of chronic myelogenous leukemia, head and neck carcinoma, and sarcoma. One of its drawbacks, however, is the development of HU resistance. To study this problem, we developed a HU-resistant human KB cell line which exhibits a 15-fold resistance to HU. The characterization of this HU-resistant phenotype revealed a gene amplification of the M2 subunit of ribonucleotide reductase (RR), increased levels of M2 mRNA and protein, and a 3-fold increase of RR activity. This HU-resistant cell line also expressed a "collateral sensitivity" to 6-thioguanine (6-TG), with a 10-fold decrease in the dose inhibiting cell growth by 50% as compared to the KB parental line. The mechanism responsible for this supersensitivity to 6-TG is believed to be related to an increasingly efficient conversion of 6-TG to its triphosphate form, which is subsequently incorporated into DNA. After passage of the resistant cells in the absence of HU, the cell line reverts. The revertant cells lose their resistance to HU and concomitantly their sensitivity to 6-TG. This phenomenon is due to the return of RR to levels comparable to that of the KB parental cell line. These observations and their relevance to cancer chemotherapy will be discussed in this paper. Our results suggest that a clinical protocol could be designed which would allow for a lower dose of 6-TG to be used by taking advantage of the increased RR activity in HU-refractory cancer patients. Two drugs which display collateral sensitivity are known as a "Ying-Yang" pair. Alternate treatment with two different Ying-Yang pairs is the rationale for the "Ying-Yang Ping-Pong" theory in cancer treatment. This rationale allows for effective cancer chemotherapy with reduced toxicity.

Introduction

HU, which is currently being used in the treatment of chronic myelogenous leukemia, head and neck carcinoma, and sarcoma (1), has also been used as a radiation potentiator in combination therapy for the treatment of non-small cell lung cancer, primary brain tumors, and cervical cancer (2). One of the complications of HU therapy is the rapid emergence of resistance. For many years this laboratory has been interested in cancer chemotherapy, mechanisms of resistance, and the development of methods to overcome this resistance. Therefore, we chose to study the mechanism of resistance of HU to better understand and overcome this obstacle. The primary target of HU is the enzyme RR. RR is the rate-limiting enzyme for the conversion of ribonucleoside diphosphates to deoxyribonucleoside diphosphates, which are precursors for DNA synthesis (3). Therefore, inhibition of RR will lead to a decrease in DNA synthesis and ultimately cell death. The structure of RR consists of four subunits, two M1 and two M2 proteins. The M1 subunit is a Mr 170,000 protein with a binding site for substrates such as CDP (4, 5). M2 (M, 88,000) contains a tyrosyl free radical, a unique protein-bound organic free radical, and non-heme iron. The iron and the tyrosyl groups are essential for the RR enzyme activity. HU is known to target on M2, interfering with the free radical of M2 and thereby inactivating RR activity (6). The molecular mechanisms of HU resistance are related to an increase in RR levels, molecular modification of the M2 subunit, and modulation in the M1:M2 subunit ratio. An understanding of the molecular mechanisms of HU resistance in human cancer cells can be used to design strategies that overcome this resistance by taking advantage of these specific changes. An example of one strategy relates to "collateral sensitivity" and the "Ying-Yang Ping-Pong" theory proposed by this laboratory (7, 8). Tumor cells which develop resistance to HU as a result of an increase in RR levels become supersensitive to 6-TG. This compound has been reported to exert its cytotoxic effects as a result of its incorporation into DNA (9). We have developed a KB cell line which has a 15-fold resistance to HU. The mechanism of resistance was shown in part to be due to a 3-fold increase in RR activity. Since this cell line also expressed collateral sensitivity to 6-TG, it became a good model in which to take advantage of increased RR levels. The elevated enzyme levels allowed for a more efficient conversion of 6-TG to its deoxy form, which resulted in the incorporation of more drug into DNA. This supersensitivity phenomenon allows for a lower dose of 6-TG to be used in HU-resistant cells, resulting in a better therapeutic index. Therefore, a greater understanding of the mechanisms of resistance and the incorporation of the collateral sensitivity phenomenon into clinical treatment protocols will enable us to achieve a more efficient use of chemotherapeutic agents and decrease drug toxicity.

Materials and Methods

Cells. KB cells from a human nasopharyngeal carcinoma line (American Type Culture Collection, Rockville, MD) were maintained at 37°C in a humidified atmosphere containing 5% CO2. These cells (KB parental, referred to in this paper as KB wild-type) were grown in RPMI 1640 supplemented with 5% dialyzed fetal bovine serum. HU-resistant cells were sequentially selected in a stepwise manner in the presence of HU. The HU-resistant cloned line was selected from colonies formed under a selection pressure of 1 mm HU. The stable cloned lines were continuously maintained in culture for 3–4 months; at 4-week intervals, samples of each cell line were banked. The stability of the resistant phenotype was then determined. Revertants of the HU-resistant cells were maintained in the absence of HU for 8 weeks. The generation times for KB wild-type, HU-resistant, and revertant cells were calculated as 18, 38, and 18 h, respectively.

Materials. HU and 6-TG were purchased from Sigma Chemical Co. (St. Louis, MO). 6-[^3H]TG was a gift from Dr. J. Arly Nelson (M. D. Anderson Cancer Center, Houston, TX) with a specific activity of 625 cpm/pmol. The restriction enzymes EcoRI, PstI, MspI, and HpaII and actin were purchased from Boehringer Mannheim Biochemicals.

Cytotoxicity Studies. The resistant cells were maintained in drug-free medium for 4 days prior to use. Cells in logarithmic growth were plated at a density of 5000 cells/ml/well in a 24-well plate (Corning Glass Works, 3686
6-TG SUGERSENSITIVITY IN A HU-RESISTANT KB CELL LINE

Coming, NY). To determine the cytotoxic effects, drugs were added to cells at different dosages. Cultures were maintained under growth conditions as described above for a period of 3 generations. The methylene blue assay was subsequently performed as described by Finlay et al. (10) and the ID$_{50}$ was determined by interpolation of the plotted data.

Clonogenic Assay. The colony-forming abilities of the KB wild-type, HU-resistant, and revertant cells in the presence and absence of HU and 6-TG were determined. The same number of logarithmic growth cells were tested with the specified concentrations of drugs and plated in 6-well plates. The number of colonies from each cell line was counted after incubation at 37°C for 8 generation times.

Preparation and Assay of RR. CDP reductase was assayed by the method of Steeper and Stuart (11) with the use of Dowex 1-borate ion-exchange chromatography. The assay mixture contains, in a final volume of 0.02 ml, 0.02 µCi [3H]CDP (52.9 mCi/mmol; DuPont New England Nuclear, Boston, MA), 3 mM dithiothreitol, 6 mM MgCl$_2$, and 10 µl of cellular extract. The incubation time for the reaction was 60 min. The reaction was linear during this process.

Cesium Sulfate Density Gradients. To study the mechanism responsible for the supersensitivity to 6-TG, nucleic acids were extracted from KB wild-type, resistant, and revertant cells which had been for one complete generation time to the specified concentration of 6-[14C]CTG. The nucleic acids were lysed in DNA/RNA extraction buffer (10 mM Tris-HCl, pH 7.6-1.1 mM EDTA-100 mM NaCl-0.5% Sarkosyl-0.5% sodium dodecyl sulfate), digested with proteinase K (6 mg/ml), and extracted with phenol/chloroform (24:1). The nucleic acids were then placed on a cesium sulfate solution (1.548 g/cm$^3$) and centrifuged in a Beckman SW 50.1 rotor at 25,000 rpm for 65 h at 20°C. The fractions collected were analyzed by methods described previously (12). The peak of radioactivity in the gradient was detected at a density of 1.42 g/ml, which represents the region of DNA.

HPLC Analysis of the Acid-soluble Fractions. Cells were incubated with 6-[14C]CTG for the indicated time and then washed with phosphate-buffered saline and extracted with 1.5 n perchloric acid. The acid-insoluble material was removed by centrifugation. The neutralized acid-soluble fractions were analyzed using HPLC with a strong anion-exchange column (Whatman Partisil SAX). The solvent used was potassium phosphate buffer, pH 6.5, with a flow rate of 1 ml/min. A step gradient system was used with 0.03 M buffer for 0—12 min followed by 0.15 M buffer for 12—52 min. At 52 min, the buffer concentration and flow rate were increased to 0.3 M and 2 ml/min, respectively. The HPLC was connected to a fraction collector and fractions were collected at 1-min intervals and used directly for scintillation counting. The amount of radioactive 6-TG metabolites in the acid-soluble fractions was calculated.

Preparation of the Probes for M1 and M2 Subunits of RR by Using PCR. The human cDNA sequence of M1 and M2 subunit of RR has been published (13). To construct the probes for the human M1 and M2 subunits, reverse transcriptase-coupled PCR was used. The polyadenylated RNAs from KB wild-type, resistant, and revertant cells and primers designed from the sequence of both subunits of human KB cells were used. The primers utilized for the M2 subunit are the 5' primer from positions 189 to 211, including the AUG site (5' ATCCGGATCCACTGTCCTCCCTCCGOT3') and the 3' primer from 1346 to 1368 including the UAA site (5'GCTTAAGGCTTATT-TAGAAGTCAGCATCCAAG 3'). The M1 subunit used a 5' primer from position 188 to 207 including the ATG (5' GCTCGAGCTCATOCATGTCGT- GATCGAAGCCA3') and a 3' primer from position 2549 to 2570 including the TGA site (5' GCAGCTGCGAGCTGAGATCCACACTA 3'). The 35-cycle PCR reaction was used as standard protocol. The PCR products consisted of single bands on 1% agarose gels and were directly ligated into TA cloning vectors, followed by the transformation of a provided competent Escherichia coli strain. The solid colonies were picked and analyzed by miniprep to verify the presence of cloned PCR product. The restriction enzymes PstI and SacI were used to cut M1 out of the vectors; the HindIII and BamHI were used for M2. After enzyme digestion, full length cDNA probes were verified by Miniprep and used as probes in the following studies.

Southern and Northern Analyses. Cells were maintained in logarithmic growth for 4 days following drug removal. Genomic DNA was extracted by the standard phenol-chloroform method. For Southern analysis, 20 μg of DNA were digested with EcoRI, PstI, MpiI, and HapII restriction endonucleases. Agarose gels (0.8%) were used for fractionation and were then transferred to nitrocellulose membranes on which hybridizations were performed. Total cellular DNA was extracted from cells and Northern blot analysis was performed. RNA was separated on a 1% agarose gel and then transferred to a Hybond-N membrane. The filter was then probed with 32P-end-labeled PCR products of human M1 or M2. The filter was exposed to X-ray film and relative amounts of mRNA were quantitated by densitometer tracing of the autoradiogram.

Antibodies and Western Blot Analysis. AD203 anti-mouse M1 monoclonal antibody and YLI/2 anti-rat tubulin polyclonal antibody, which cross-reacts with the human M2 subunits of RR (14—16), were purchased from Accurate Chemical & Scientific (Westbury, NY). Cellular extracts were analyzed with 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis by loading equivalent amounts of protein. After electrophoresis, the proteins were transferred to a nitrocellulose membrane. The membrane was then incubated with AD203 anti-mouse M1 monoclonal antibody or YLI/2 anti-rat M2 antibody. Goat anti-mouse IgG conjugated with alkaline phosphatase (Sigma) was used as secondary antibody to M1; rabbit anti-rat IgG was used for M2. The quantitative difference of M1 and M2 between KB wild-type and resistant cells was determined after development with enhanced chemiluminescence (Amersham, Arlington Heights, IL).

Results

Effect of HU on KB Wild-Type, HU-resistant, and Revertant Cells. The sensitivity of KB wild-type, HU-resistant, and revertant cells to HU is shown in Table 1. The ID$_{50}$ values of HU for wild-type, HU-resistant, and revertant cells are 0.3, 4.5, and 0.3 mM, respectively. The RR sensitivity to HU remained constant in the three cell lines. Therefore, the possibility of a major alteration of the RR enzyme structure appears to be unlikely. Since the doubling times of these cell lines differ, these results were obtained from experiments performed using cells standardized by a period of 3 generations. The HU-resistant cells exhibited approximately 15-fold more resistance to the cytotoxic effects of HU than KB wild-type cells.

Colony-forming abilities for KB wild-type, HU-resistant, and revertant cells exposed to different concentrations of HU are shown in Fig. 1a. HU-resistant cells present equal colony-forming ability as compared to KB wild-type and revertant cells in the absence of drug. However, the HU-resistant cells exhibit an approximately 20% higher colony-forming ability than KB wild-type and revertant cells in the presence of HU. The ID$_{50}$ of HU is 4.5 mM in HU-resistant cells and 0.3 mM in KB wild-type and revertant cells.

To determine RR activity of each cell line, cell extracts were prepared and RR activity was determined utilizing [14C]CDP as a substrate. The HU-resistant cells exhibited a 3-fold elevation of RR activity over KB wild-type cells (Table 1). The revertant cells, which lost their resistance to HU, had approximately the same level of RR activity as the KB wild-type cells. To determine the possibility of an alteration of the RR enzyme, enzyme activity in KB wild-type, KB-resistant, and revertant cell preparations was determined in the presence of increasing concentrations of HU (data not shown). These data revealed no significant difference in the sensitivity of RR to HU in these three cell lines.

Characterization of the Mechanism Responsible for the HU-resistant Phenotype. Northern blots probed with a 2.4-kilobase PCR product of full length human M1 cDNA showed that HU-resistant cell lines express slightly increased levels of M1 mRNA over that of the KB wild-type and revertant cells (Fig. 2). However, the significance

Table 1 Effect of HU on KB wild-type, HU-resistant, and revertant cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>ID$_{50}$ (mm)</th>
<th>Relative RR activity$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>KB wild-type</td>
<td>0.323 ± 0.059</td>
<td>1.0</td>
</tr>
<tr>
<td>KB HU-resistant</td>
<td>4.5 ± 0.37</td>
<td>3.13 ± 0.15</td>
</tr>
<tr>
<td>KB HU-revertant</td>
<td>0.333 ± 0.20</td>
<td>1.16 ± 0.003</td>
</tr>
</tbody>
</table>

$^a$ Enzyme activity was determined as described under “Materials and Methods” and was expressed as activity relative to the KB wild type.
of this increase has not been determined. Probing Northern blots with 1.1-kilobase M2 cDNA indicated that the resistant cell line possessed elevated levels of M2 mRNA (Fig. 2). Two bands at approximately 3.4- and 1.6-kilobase mRNA were also detected. The revertant cell line exhibited the same level of M2 mRNA as KB wild-type. Table 2 shows a 10-fold elevation of M2 message levels in the HU-resistant line compared to the KB wild-type cell line determined by densitometric measurements.

The restriction enzymes EcoRI, PstI, MspI, and HpaII were used on the DNA extracted from each cell line. Southern blots examined with specific M1 probes showed no difference in the banding patterns or the band intensities in KB wild-type, HU-resistant, and revertant cells. However, HU-resistant cells digested with MspI and screened with an M2 probe clearly showed different banding patterns and band intensities (Fig. 3). Increased band intensities were observed from MspI digestion but not HapII, which indicates that the M2 gene has no increase in methylation but does have gene amplification. Densitometric measurements indicated a 6-fold increase in the gene for the M2 subunit in HU-resistant cells when compared to KB wild-type and revertant cells.

The levels of M1 and M2 proteins were determined by Western blot analysis and the results are shown in Fig. 4. The Western blot studies reveal that production of M1 subunit has no significant difference in KB wild-type, HU-resistant, and revertant cells. However, corresponding M2 proteins were increased 3-fold in our HU-resistant cells when compared to the KB wild-type and revertant cells.

**Supersensitivity of HU-resistant Cells to 6-Thioguanine.** The sensitivity of KB wild-type, HU-resistant, and revertant cells to 6-TG is shown in Table 2, with 6-TG ID50 values of approximately 0.1, 0.01, and 0.1 μM, respectively. Therefore, this resistant cell line exhibited a 10-fold increase in sensitivity to 6-TG. Colony-forming abilities for KB wild-type, HU-resistant, and revertant cells exposed to different concentrations of 6-TG are shown in Fig. 1b. Resistant cells exhibit slightly less colony-forming ability than did KB wild-type and revertant cells when exposed to one ID50 of 6-TG. When exposed to the same concentration of 6-TG, HU-resistant cells show approximately 10-fold less colony-forming ability than KB wild-type and revertant cells. Therefore, the HU-resistant cells are 10-fold more sensitive to 6-TG in the cytotoxicity study, as well as in the colony-forming assay.
Table 2 6-TG collateral sensitivity in KB HU-resistant cell lines

<table>
<thead>
<tr>
<th></th>
<th>ID50 (μM)</th>
<th>AS*</th>
<th>DNA*</th>
<th>RNA*</th>
</tr>
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<tbody>
<tr>
<td>KB wild-type</td>
<td>0.116 ± 0.020</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>KB HU-resistant</td>
<td>0.011 ± 0.003</td>
<td>1.5</td>
<td>11.0 ± 0.37</td>
<td>2.8 ± 0.175</td>
</tr>
<tr>
<td>KB HU-revertant</td>
<td>0.112 ± 0.016</td>
<td>1.12</td>
<td>0.9 ± 0.003</td>
<td>0.9 ± 0.21</td>
</tr>
</tbody>
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* Acid-soluble (AS), DNA, and RNA values were determined at 0.01 μM as described under "Materials and Methods" and are expressed as the amount of 6-[14C]thioguanine incorporated into the nucleic acid relative to the KB wild type. The absolute values for KB wild-type DNA and RNA are 188 and 121 cpm/10⁷ cells, respectively.

To understand the mechanism of 6-TG supersensitivity, the incorporation of 6-[14C]TG into KB wild-type, HU-resistant, and revertant cells was examined. Each cell line was exposed to 6-TG for one generation time at the indicated ID₅₀. Cells were lysed in DNA/RNA extraction buffer, digested, extracted, and layered onto a cesium sulfate density gradient as described under "Materials and Methods." The density gradients revealed two peaks of radioactive nucleic acids. The first peak (fractions 4–6), with a density of 1.42 g/cm³, is alkali stable (DNA). The second peak (fractions 36–38), with a density of 1.65 g/cm³, is alkali labile (RNA). The majority of radioactivity was observed in the DNA peak with minor incorporation into the RNA peak. This was the same for several different concentrations of 6-TG. As shown in Fig. 5, the radioactivity incorporated into DNA was higher in the HU-resistant cells compared with KB wild-type or the revertant cells. This is most significant when the 0.01 μM concentration of 6-TG is utilized. The relative amounts of radioactivity incorporated in the DNA and RNA fractions in each cell line after exposure to 0.01 μM 6-[14C]TG are shown in Table 2. The incorporation of 6-[14C]TG was approximately 10-fold greater in HU-resistant cells versus KB wild-type and revertant cells. When examined by HPLC, the radioactivity in acid-soluble fractions from resistant cells was approximately 1.5-fold higher than the radioactivity in the acid-soluble fractions from KB wild-type and revertant cells (Table 2).

Discussion

HU exerts its cytotoxic effect by binding to the M2 subunit of RR and rendering the enzyme inactive. Once the RR activity is inhibited by HU, DNA synthesis will be blocked and the cells will subsequently be killed. In this study, we report that KB HU-resistant cells exhibit a 15-fold increase in resistance to HU as compared to the KB wild-type and revertant cells. Using the traditional 72-h cytotoxicity study, we observed an apparently greater increase in resistance from the HU-resistant cell line due to differences in doubling time (data not shown). Therefore, these results have been standardized to the same number of cell cycles since the generation times are unequal.

Using clonogenic assays, we compared the colony-forming ability of each cell line after treatment for one full generation time with HU at different fold increases of ID₅₀. More than 50% of cells formed colonies in the HU-resistant cell line, while less than 50% of cells formed colonies in the KB wild-type and revertant cell lines. These results are consistent with the 6-TG collateral sensitivity data, which showed a 15-fold increase in resistance to HU in the HU-resistant cell line.

Fig. 4. Western blots of M2 and M1 in KB wild-type, HU-resistant (Resist.), and HU-revertant (Revert.) cells. Crude cell extracts (50 μg) of RR protein were loaded in each lane. The molecular weight in thousands (kD) indicates the monomer of each subunit.

Fig. 5. 6-Thioguanine incorporation into DNA and RNA of KB wild-type, HU-resistant, and HU-revertant cells. Cells were treated with different concentrations of 6-[14C]TG and applied to cesium sulfate density gradients as described under "Materials and Methods." Total radioactivity was calculated from DNA and RNA fractions. Points, means from 3 independent studies; bars, SE; Conc., concentration.
colonies when the HU-resistant cells were treated with one ID$_{50}$ of HU (Fig. 1a). The revertant cells, however, lost the resistant phenotype in the absence of HU and showed the same colony-forming ability as KB wild-type cells.

It has been suggested that this increased resistance is caused by the increase in RR activity (12, 14, 17). In our study, we observed a 3-fold increase of RR activity in the HU-resistant cell line. The alteration of the RR enzyme has been found to decrease its sensitivity to HU (18).

In our report, HU inhibited the RR enzyme activity of KB wild-type, HU-resistant, and revertant cells in a similar fashion (Table 1). Since a crude cell preparation of RR was used in this study, the data may not reflect some conditions in vivo.

Earlier reports have shown that the molecular mechanism of HU resistance relates to an increased level of M2 mRNA (17). However, another study reported that the highly drug-resistant cells may also contain an alteration of the $M_1$ gene expression (19). From Northern blot analysis, we observed that our resistant cells contained increased levels of M2 mRNA. However, our study could not show a significant alteration in the levels of $M_1$ gene expression as in studies described by Hurta and Wright (19). The differences in our observation could be explained if an increase in expression of M1 can be observed only when cells with extremely high levels of HU resistance are used or if significant alteration of the $M_1$ gene occurs only in murine cells. These questions must be explored further. Northern analysis displayed two M2 transcripts from KB wild-type, HU-resistant, and revertant cells, one at 3.4 kilobases and the other approximately 1.6 kilobases. This observation is believed to be caused by additional polyadenylation sites and is consistent with previous reports (14, 17).

Southern blot analysis of KB wild-type, HU-resistant, and revertant cell DNA digested with EcoRI, PstI, MspI, and HpaII restriction endonucleases and probed with the M1 cDNA showed no difference in banding patterns. This result suggests that this resistant phenotype is not related to $M_1$ gene amplification. However, when the same DNA was probed with M2 cDNA, there was a selective amplification of certain gene fragments. Moreover, the changes can be observed only in resistant cells digested with MspI but not in those digested with HpaII, strongly indicating an increase in gene amplification rather than in methylation of the DNA gene.Earlier reports have shown that the M2 gene has been mapped to human chromosome 2 and pseudogenes were found in mouse and human DNA (20, 21). Whether our HU-resistant cell line presents pseudogenes, double minutes, or other closely related genes requires further exploration.

From Western blot analysis, our result indicates that the M2 protein was 3-fold overproduced in the HU-resistant cell line when compared to KB wild-type and revertant cell lines. The comparison of M1 protein in KB wild-type, HU-resistant, and revertant cells revealed no significant changes. The increased M2 protein level strictly correlated with RR enzyme activity in the HU-resistant cell line. It has been known that the mouse HU-resistant cells cultured in the presence of HU can promote the M1 and M2 protein levels and activity without an increase in the corresponding mRNA. This phenomenon suggests that HU probably modulates RR expression at the posttranscriptional level (14). In our study, all the assays were performed after 4 days without drug to avoid any regulation from HU. Nevertheless, the degree of alteration in mRNA and protein is different than the gene amplification, suggesting the possibility that posttranslational or posttranscriptional regulation might be involved in this HU-resistant line (17).

In summary, from our results, the HU-resistant cell line exhibited a 3-fold elevated level of RR activity. Western blot analysis indicated a comparable increase in the M2 subunit protein level. Northern and Southern blot analyses indicated that this HU-resistant cell line has increased levels of mRNA and gene amplification of the M2 subunit. Since revertant cells had levels of protein, DNA, and RNA similar to those of the KB wild type, we conclude that these alterations in mRNA and gene amplification are directly involved in the HU resistance phenotype.

It has been known that 6-TG is metabolized to the deoxynucleotide form by RR and consequently incorporated into DNA. Once 6-TG is incorporated into DNA, it will impair the DNA function (9, 22, 23). Our results clearly indicate that HU-resistant cell lines exhibit supersensitivity to 6-TG in cytotoxicity studies and colony-forming assays (Table 2). The mechanism of this supersensitivity is related to the increase of RR activity in the resistant cells. The elevation of RR activity allows for a substantial amount of 6-TG to be converted to deoxynucleotide and subsequently incorporated into DNA, causing supersensitivity. The cesium sulfate study confirmed this hypothesis by revealing increased amounts of 6-TG in the DNA of the HU-resistant cell line. The revertant cells, however, revealed 6-TG incorporation comparable to the KB wild type.

On the basis of the results of 6-TG incorporation studies shown above, we propose using the collagenatically sensitive agents, such as 6-TG, to kill cells that display HU resistance. This new principle should be used to design a new clinical protocol in combination chemotherapy.

References


19. Hurta, R. A. R., and Wright, J. A. Mammalian drug resistant mutants with multiple 
gene amplification: genes encoding the M1 component of ribonucleotide reductase, 
the M2 component of ribonucleotide reductase, ornithine decarboxylase, p53, the 
H-subunit of ferritin and the L-subunit of ferritin. Biochim. Biophys. Acta, 1087: 
20. Brissenden, J. E., Caras, I., Thelander, L., and Francke, U. The structural gene for the 
M1 subunit of RR maps to chromosome 11, band p15, in human and to chromosome 
21. Yang-Feng, T. L., Barton, D. E., Thelander, L., Lewis, W. H., Srinivasan, P. R., 
and Francke, U. Ribonucleotide reductase M2 subunit sequences mapped to 
four different chromosomal sites in humans and mice: functional locus identified 
by its amplification in hydroxyurea-resistant cell lines. Genomics, 1: 77–86, 
1987.
22. Pan, B. F., and Nelson, J. A. Characterization of the DNA damage in 6-thioguanine-
sequence-specific protein-DNA interactions by incorporation of 6-thioguanine: cleav-
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