Combination Cytotoxic Effects of cis-Diamminedichloroplatinum(II) and 5-Fluorouracil with and without Leucovorin against Human Non-Small Cell Lung Cancer Cell Lines

Chun-Ming Tsai,¹ Shu-Huei Hsiao, Carolin M. Frey, Kuo-Ting Chang, Reuy-Perng Perng, Adi F. Gazdar, and Barrnett S. Kramer

Chest Department, Veterans General Hospital-Taipei, Taipei, Taiwan; C-M. T., S-H. H., K-T. C., R-P. P.; Surveillance [C. M. F.] and Early Detection and Community Oncology [B. S. K.] Programs, National Cancer Institute, Bethesda, Maryland 20892; and Simmons Cancer Center, University of Texas Southwestern Medical Center, Dallas, Texas 75235 [A. F. G.]

ABSTRACT

Both cisplatin (CDDP) and leucovorin (LV) have been shown to enhance cytotoxicity of 5-fluorouracil (FUra) against murine and human neoplasms by increasing intracellular reduced folate concentrations. We were interested in their use in a combination to inhibit non-small cell lung cancer (NSCLC) cell growth and therefore conducted an in vitro study to investigate the cytotoxic activities of combinations of CDDP plus FUra, with and without LV (20 μM), against seven NSCLC cell lines. A tetrazolium assay with application of the classical isobole method was used to test drug combinations. We found that LV enhanced FUra but not CDDP cytotoxicity and that the degree of enhancement was negatively correlated with the effect of FUra. There was an overall additive combination effect of CDDP plus FUra, although there may be synergy at higher effect levels. There was synergy to a combination of CDDP, FUra, and LV, presumably primarily related to the synergistic effects of adding LV to FUra. In summary, LV and CDDP enhanced FUra cytotoxicity in a complementary fashion and there was clear synergy of a combination of CDDP, FUra, and LV against a panel of NSCLC cell lines. Our in vitro results provide a rationale for controlled clinical studies of this three-drug regimen in patients with NSCLC.

INTRODUCTION

Few malignancies have been as frustrating to medical oncologists as has NSCLC.¹ This is compounded by the high incidence of the disease, its frequently disseminated stage at presentation, and a median survival that is best measured in weeks or months for all but the few patients presenting with highly localized and surgically accessible disease. Most chemotherapeutic drugs, both single agents and combinations of drugs, are not particularly active against this tumor. To improve the likelihood of response, response duration, and survival, it is clear that more active drugs or drug combinations are required. FUra, a fluorinated pyrimidine widely used in the treatment of a variety of solid tumors, when used alone produces an objective response in 14–18% of patients with NSCLC (1). FUra has also been combined with other chemotherapeutic agents with response rates of 33–58% (1). CDDP, another agent with broad activity against a variety of human tumors, has been shown to potentiate FUra cytotoxicity against both murine and human neoplasms (2–4). When studied against L1210 leukemia in mice, CDDP plus FUra, each of which alone only modestly prolonged median survival, resulted in a 60% disease-free long-term survival (2). Sridhar et al., using a human pancreatic tumor cell line, have demonstrated that CDDP followed after 1 or 24 h by FUra results in additive tumor inhibition. However, the reversed sequence of the drugs, with exposure to CDDP following FUra, produces an antagonistic effect resulting in significantly less inhibition of tumor growth than exposure to FUra alone (3). Using a human ovarian carcinoma line, Scanlon et al. (4) have also shown that low concentrations of CDDP followed by FUra are more cytotoxic than the reverse sequence or either drug alone. Their in vitro studies have demonstrated that an initial 30-min exposure to CDDP increased the intracellular pools of CH₂THF and tetrahydrofolate 2.5-fold and suggested the observed enhanced effect of FUra to be due to increased intracellular reduced folate concentrations following the administration of CDDP. At the clinical level, several uncontrolled phase II studies (5–8) and randomized trials (9, 10) have been reported primarily in colon, head and neck, and genitourinary tract cancers, as well as NSCLC. The results, although promising in some studies, do not allow definitive claims regarding the clinical applicability of the synergy concept.

Recently, attempts to improve the therapeutic effectiveness of FUra have focused on the scientific principles of biochemical modulation: alteration of tumor cell metabolism to produce selective enhancement of cytotoxicity. One of the current biochemical modulation approaches is the use of LV to enhance the activity of FUra. The rationale for this combination is based upon the observation that reduced folates can increase FUra cytotoxicity by forming and maintaining a stable ternary complex with the FUra metabolite FdUMP and the target enzyme TS (11, 12). The stabilized complex theoretically increases the block in DNA synthesis, thus enhancing cytotoxicity. Clinically, this concept has been successfully applied in the treatment of patients with a variety of cancers, including colon, breast, and stomach cancers. Several randomized studies, primarily in colorectal cancer, have reported improved response rates in patients treated with FUra and LV compared with patients treated with FUra alone (9, 13–16) and some studies have reported improved survival of patients treated with FUra and LV (15, 16).

Both CDDP and LV interact with FUra by increasing intracellular reduced folate levels and thereby the inhibition of TS. Therefore, the addition of LV and CDDP to FUra may increase the activity of FUra. Recently, nonrandomized clinical trials of FUra combined with CDDP and LV have been reported to demonstrate substantive rates of objective regression in patients with advanced head and neck tumors (17–20). Previously, they have reported that LV can enhance fluorinated pyrimidine-induced cytotoxicity against lung cancer cell lines (21). Thus far, there is no laboratory evidence to confirm the superiority of this 3-drug regimen. We now report the combination effects of CDDP and FUra with and without LV in a panel of NSCLC cell lines.

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: NSCLC, non-small cell lung cancer; CDDP, cisplatin [cis-diamminedichloroplatinum(II)]; LV, leucovorin [5-formyltetrahydrofolate]; FUra, 5-fluorouracil; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TS, thymidylate synthase; FdUMP, fluorodeoxyuridine monophosphate; CH₂THF, 5,10-methylenetetrahydrofolate; RFPMI 1640 supplemented with 5% fetal bovine serum; IC₅₀, IC₅₀, and IC₅₀, concentrations of drug which produced 30, 50, and 70% reduction in control absorbance, respectively; FL, FUra + LV; CL, CDDP + LV; CF, CDDP + FUra; CCL, CDDP + FUra + LV; FL [L] and CFL [L] data were calculated by using the wells with LV [20 μM] alone as control. They were used to express the cytotoxic effects of FUra, CDDP, and FUra in the presence of LV, respectively.
MATERIAL AND METHODS

Cell Lines

Seven NSCLC cell lines (adenocarcinomas NCI-H23, NCI-H838, NCI-H1437; squamous cell carcinoma NCI-H226; adenosquamous cell carcinoma NCI-H125; large cell carcinomas NCI-H460 and NCI-H1334) were studied. All lines were established from previously untreated patients and were grown in RPMI 1640 ( Gibco supplemented with 10% fetal bovine serum and adapted in R5 for more than 6 months before this study.

Trypsin-EDTA was used to make single cell suspensions after cultures were washed with phosphate-buffered saline. Trypsin was naturalized with R5 and cells were resuspended in R5 for cytotoxic assays. Cells were then plated at the appropriate seeding density, individualized for each cell line, in 140 μm medium into 96-well microtiter plates and incubated for 24 h at 37°C in a 5% CO2 atmosphere in order to allow recovery from trypsinization before cytotoxic assays began. All cell lines were in logarithmic phase of growth at the time of the MTT assay.

Study Design and MTT Assay

There were 17 plates prepared for each cell line per test. One of the plates was used to determine the dose-response relation of LV alone at concentrations of 5,10, 20, 30, 50, 100, 300, 1000, and 3000 μM. Previous studies in murine sarcoma 180, human carcinoma Hep-2, and murine leukemia L1210 suggested that maximal stabilization of the ternary complex of reduced folate with FdUMP and TS and potentiation of FUra cytotoxicity were achieved at a total LV concentration of 20 μM (22). We have previously reported that this clinically achievable level of LV can enhance cytotoxic effects of FUra and 5-fluorodeoxyuridine in a panel of human lung cancer cell lines (21). Therefore, all assays using LV in combination with CDDP and FUra were performed at 20 μM only. The remaining 16 plates were divided into a CF group and a CFL group, 8 plates of each (Fig. 1). Over these plates, each had one 8-well blank column with media alone and two 8-well control columns with cells but no drug. For the 8 plates of CF group, each had a 8-well column with single agent CDDP. The remaining 8-well columns contained a constant concentration of CDDP plus increasing concentrations of FUra in 0.5- or 1-log increments from column to column. Over a series of 7 plates, the concentration of CDDP was increased in 0.5-log increments from plate to plate. An eighth plate was used for single drug controls with increasing concentrations of single agent FUra from column to column. Each drug was tested to cover the entire dose-response curves whenever possible, as determined by preliminary testing. For the 8 plates of the CFL group, the study design was the same as those of CF group but 20 μM LV was added to every drug-testing well. In addition, each plate had one 8-well LV control column with cells and single agent LV (20 μM). With this design, 16 survival curves were generated from every set of 8 plates.

The day after the cells were plated into the microtiter wells, medium and drugs were added as follows. Sixty μl of R5 were added to no-drug control wells; 40 μl of R5 and 20 μl of drug were added to single drug wells(CDDP, FUra, LV); 20 μl of R5 and 20 μl of each drug were added to 2-drug combination wells (CL, FL, CF) and 20 μl of each drug were added to 3-drug combination wells (CFL). LV and FUra were added 1 h and 30 min before FUra, respectively. FUra (Roche Laboratories, Nutley, NJ) was diluted with R5; LV (Lederle, Inc., Wayne, N.J.) and CDDP (David Bull Laboratories, Mulgrave, Victoria, Australia) were dissolved with distilled water and phosphate-buffered saline, respectively, to 100 μm solution and then subsequently diluted with R5.

After addition of drugs, the microtiter plates were incubated for 96 h. Cell survival was then determined by a MTT colorimetric assay (23). The percentage of control absorbance was considered to be the surviving fraction of cells and the IC50, IC50, and IC70 values were calculated for all the plates using the wells with cells but no drug as control. For the plates of the CFL group, the values were additionally calculated using the wells with single agent LV as control. Data points calculated from the testing wells of CL, FL, and CFL regimens by the latter way represented the cytotoxic effects of CDDP, FUra, and CDDP + FUra in the presence of LV. They were termed as CF [L], CFL [L], and CFL, respectively, for this evaluation. The experiment was performed in quadruplicate on each of the cell lines. The IC50, IC50, and IC70 values of the four tests as well as their mean values were calculated.

Data Analysis

LV Cytotoxicity and Its Effects on FUra and CDDP. The Wilcoxon signed rank test was applied to analyze the effect of LV alone (20 μM) and it was used to compare the cytotoxic effects of CDDP and FUra with and without LV (FUra versus FL, FUra versus FL; CDDP versus CL, CDDP versus CFL). To evaluate the degree of enhancement of LV on the cytotoxic effects of FUra and CDDP between two different effect levels, the ratio of IC values without LV to those in the presence of LV (IC[L]/IC[L]) and CIC[L]/CIC[L]) were also compared by the Wilcoxon signed rank test. All tests are two sided and significance is assumed for P < 0.05.

CF Cytotoxicity and the Effect of LV on It. The classical isobole method of Berenbaum (24) was used to determine the in vitro combination effects of CDDP and FUra with and without LV. With this method, data points above the straight diagonal line of additive effect in the isobole signify antagonism and those below the diagonal signify synergy.

We used the combination index (CI) to compare the cytotoxic effects of CF, CFL, and CF[L]. The CI was defined as the sum of the relative doses (e.g., IC50 units) of each drug which yield an isoeffect (e.g., an inhibition of 0.3) when added together.

\[ CI = \frac{\text{Dose of CDDP}}{\text{IC value of CDDP}} + \frac{\text{dose of FUra}}{\text{IC value of FUra}} + \frac{\text{dose of LV}}{\text{IC value of LV}} \]

Here, dose of CDDP, dose of FUra, and dose of LV (zero μM for CF and CFL) 20 μM for CFL) added together yield an isoeffect (e.g., 30%) cell kill (For example, the IC50 values of single agent CDDP, FUra, and LV of the cell line NCI-H226 were 2.2, 38, and 274 μM, respectively. The same effect could be achieved by adding CDDP 1.06 μM, FUra 4.0 μM, and LV 20 μM together. Therefore, the CI in this experimental point was 0.66 = [1.06/2.2] + (4/38) + (20/274)]. Each experiment generates a set of CI values for a particular effect level because of the fact that there are multiple drug concentrations within the assay range that achieve the same isoeffect. Four experimental replications provide quadruplicate CI values for each member of the set. These four values were averaged to produce a single set of CI values for a particular cell line, drug combination, and effect level. The mean CI value for this set is reported as the summary measure.

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Fig. 1. Study design, I-7, drug concentrations of CDDP; a-g, drug concentrations of FUra; L, leucovorin, 20 μM/well (see "Materials and Methods" for detail).

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Upper and lower bounds of 1.05 and 0.95 were selected as being of interest so that mean CI values greater than 1.05 or less than 0.95 are interpreted as being suggestive of antagonism and synergy, respectively. Sign tests were performed on each set of CI values, in order to formally evaluate whether synergy or antagonism was evident for particular cell line, drug combination and effect levels. In addition, Wilcoxon signed rank tests were computed to evaluate whether significant differences in the cell line means occurred (a) between effect levels, (b) between drug regimens, and (c) as compared to a null hypothesized CI of 1. All tests are two-sided and significance is assumed if P < 0.05.

RESULTS

LV Alone (20 μM) Had a Minimal, but Statistically Significant Cytotoxicity. The cytotoxicity of LV was dose dependent. The mean IC_{50} (± SEM) of the seven cell lines was 863 ± 36 μM (range, 590–1100 μM). The mean absorbances (± SEM) of the control wells was 1.074 ± 0.061 and 0.994 ± 0.02, respectively. A Wilcoxon signed rank test applied to survival in the presence of LV as reported in the second column of Table 1 indicates these are significantly less than 100% (P < 0.03). Therefore, 20 μM LV had a slight, but statistically significant inhibitory effect on cell growth (mean percentage of inhibition, 5.9 ± 2.1) (Table 1).

LV Enhanced FUra Cytotoxicity but Not CDDP Cytotoxicity. The cytotoxic effects of FUra and CDDP without and with LV are listed in Table 1. In vitro, FL and CL were statistically significantly more cytotoxic than FUra and CDDP, respectively, as compared by the values of mean IC at the three observed effect levels (P < 0.05). However, when the effect of LV was segregated by calculating the data with LV alone as control, IC_{50}FUra versus IC_{50}CL were statistically significant at 30 and 50% effect levels (P = 0.018 and 0.028, respectively) but neither IC_{50}FUra versus IC_{50}CL at 70% effect level nor IC_{50}CDDP versus IC_{50}CL at any of the three effect levels was significant. Thus, LV enhanced FUra at lower and middle effect levels but had no effect on the cytotoxicity of CDDP. The values (mean ± SEM) of the concentration ratio of F/F_CL at 30, 50, and 70% effect levels were 3.69 ± 0.96, 2.06 ± 0.44, and 1.12 ± 0.15, respectively. LV enhanced FUra cytotoxicity much more at the 30 and 50% effect levels and very little at the 70% effect level, yielding a negative correlation between the effect of FUra and the degree of LV enhancement (R = -0.998, P < 0.02 by simple correlation) (Fig. 2). Fig. 2 also shows that LV had no effect on CDDP cytotoxicity for the observed effect levels. All the mean C/C_{50} values were close to 1.0.

CF Regimen Tended to Be Additive. The mean CI values of CF, CFL, and CF/L are shown in Table 2. The combination effects of the CF regimen was more cytotoxic at a higher (70%) effect level (Fig. 3). However, the degree of LV enhancement (R = -0.998, P < 0.02 by simple correlation) (Fig. 2). Fig. 2 also shows that LV had no effect on CDDP cytotoxicity for the observed effect levels. All the mean C/C_{50} values were close to 1.0.

Table 1: Effects of leucovorin (LV, 20 μM) alone and on cytotoxic effects of FUra and CDDP against non-small cell lung cancer cell lines at different effect levels:

<table>
<thead>
<tr>
<th>Cell line</th>
<th>LV (20 μM) alone (% control absorbance)</th>
<th>Effect level (%)</th>
<th>FUra concentration (μM)</th>
<th>CDDP concentration (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F/Lb</td>
<td>F/Lb*</td>
<td>C/Lb</td>
</tr>
<tr>
<td>H23</td>
<td>97.4 ± 0.3</td>
<td>30</td>
<td>2.93 ± 0.37</td>
<td>2.15 ± 0.29</td>
</tr>
<tr>
<td>50</td>
<td>27.3 ± 7.6</td>
<td>2.38 ± 0.25</td>
<td>2.15 ± 0.31</td>
<td>2.13 ± 0.29</td>
</tr>
<tr>
<td>70</td>
<td>210 ± 24</td>
<td>220 ± 33</td>
<td>4.40 ± 0.55</td>
<td>4.45 ± 0.59</td>
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<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>H125</td>
<td>101.0 ± 0.7</td>
<td>30</td>
<td>0.29 ± 0.04</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>50</td>
<td>1.27 ± 0.38</td>
<td>0.04 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>70</td>
<td>66.5 ± 27.1</td>
<td>0.16 ± 0.04</td>
<td>0.43 ± 0.03</td>
<td>0.42 ± 0.02</td>
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<tr>
<td></td>
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</tr>
<tr>
<td>H226</td>
<td>95.6 ± 0.7</td>
<td>30</td>
<td>42.3 ± 9.2</td>
<td>2.23 ± 0.27</td>
</tr>
<tr>
<td>50</td>
<td>198 ± 66</td>
<td>6.43 ± 0.67</td>
<td>6.20 ± 0.83</td>
<td>4.98 ± 0.52</td>
</tr>
<tr>
<td>70</td>
<td>930 ± 83</td>
<td>870 ± 88</td>
<td>13.4 ± 1.4</td>
<td>12.3 ± 1.0</td>
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<td></td>
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<td></td>
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<tr>
<td>H460</td>
<td>83.4 ± 1.1</td>
<td>30</td>
<td>0.91 ± 0.12</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>50</td>
<td>1.64 ± 0.17</td>
<td>0.37 ± 0.02</td>
<td>0.44 ± 0.02</td>
<td>0.36 ± 0.05</td>
</tr>
<tr>
<td>70</td>
<td>3.24 ± 0.43</td>
<td>1.68 ± 0.26</td>
<td>0.80 ± 0.03</td>
<td>0.69 ± 0.04</td>
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<td></td>
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<tr>
<td>H838</td>
<td>96.4 ± 0.6</td>
<td>30</td>
<td>0.61 ± 0.06</td>
<td>1.71 ± 0.16</td>
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<tr>
<td>50</td>
<td>8.10 ± 0.67</td>
<td>0.19 ± 0.06</td>
<td>3.90 ± 0.19</td>
<td>3.32 ± 0.31</td>
</tr>
<tr>
<td>70</td>
<td>107 ± 9</td>
<td>111 ± 17</td>
<td>7.40 ± 0.42</td>
<td>6.60 ± 0.34</td>
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<td></td>
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<tr>
<td>H1334</td>
<td>93.0 ± 0.8</td>
<td>30</td>
<td>1.09 ± 0.07</td>
<td>1.50 ± 0.28</td>
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<tr>
<td>50</td>
<td>4.08 ± 0.39</td>
<td>0.63 ± 0.06</td>
<td>4.36 ± 0.84</td>
<td>3.35 ± 0.39</td>
</tr>
<tr>
<td>70</td>
<td>660 ± 130</td>
<td>788 ± 307</td>
<td>14.7 ± 3.5</td>
<td>12.6 ± 1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1437</td>
<td>91.6 ± 1.2</td>
<td>30</td>
<td>0.02 ± 0.01</td>
<td>1.39 ± 0.09</td>
</tr>
<tr>
<td>50</td>
<td>4.13 ± 0.80</td>
<td>1.47 ± 1.07</td>
<td>6.43 ± 0.13</td>
<td>5.25 ± 0.76</td>
</tr>
<tr>
<td>70</td>
<td>420 ± 2.25</td>
<td>568 ± 94</td>
<td>14.9 ± 0.4</td>
<td>14.6 ± 0.7</td>
</tr>
</tbody>
</table>

*Expressed as percentage of control absorbance.

b Data obtained by using the wells with LV in the presence of LV as control. They were used to express the cytotoxic effects of FUra and CDDP in the presence of LV.

c Hypothesized CI of 1.0. All tests are two-sided and significance is assumed if P < 0.05.
LV Enhanced CF Cytotoxicity through the Effect Levels Studied. The CFL regimen demonstrated synergy consistently throughout the effect levels of this study (P < 0.05 at each effect level by Wilcoxon signed rank test comparing cell line CI means to 1) (Table 2). Note, however, that the sign tests evaluating departures from additivity for each cell line separately are generally nonsignificant at the 30% effect level but do achieve significance at the high effect levels. This is likely due to low statistical power with the smaller sample sizes contributing to the means at the lowest effect level for which greater cytotoxicity generates fewer data points. Hence, the sign tests should be interpreted in conjunction with the interval measure of 0.95–1.05. In addition, for each of the three effect levels, the CFL means were significantly lower than those for CF (Fig. 3).

**DISCUSSION**

Synergy between drugs in a multiagent regimen could be of potential clinical utility if the supraadditive effects were greater for tumor cells than for normal critical tissues. There is still controversy over which method is best for detecting true in vitro synergy between drug combinations (25). The most commonly used method is the classic isobologram (24). Others have proposed a modification of this technique for combinations of agents with dissimilar dose-response curves (26). It uses an envelope of additivity, or "envelope of uncertainty" to
try to account for uncertainty surrounding the possibilities that drug actions are identical or completely independent. Using the semiautomated tetrazolium-based colorimetric (MTT) assay, we have applied the concept of an envelope of additivity in a previous publication on etoposide-cisplatin interactions (27). We have also used both the classic isobologram and the Steel modification to determine the impact of schedule of administration on the combination effects of methotrexate and FURA (28). However, based on the concept of the envelope of additivity, it is difficult to develop an objective analysis to compare the combination effects of different multiagent regimens. Therefore, in this report, we used the classic isobole method and CI values to analyze and compare the different combination effects between the regimens tested.

In this study, using a panel of 7 human NSCLC cell lines we found that LV enhanced FURA at lower effect levels; there was an overall additive combination effect of CDDP plus FURA, although there may be synergy at higher effect levels. There was synergy at a combination of CDDP + FURA + LV, presumably primarily related to the synergistic effects of adding LV to FURA. Although the interaction between CDDP and FURA in the presence of LV was less active at low and middle effect levels (but not at a relatively higher effect level), this effect was masked by a much higher degree of enhancement of LV on the cytotoxicity of FURA.

It has been shown both in vitro and in vivo that the cytotoxicity of FURA is increased by LV (11-16, 21). FURA penetrates rapidly into cells where it is converted enzymatically to FdUMP, which is a potent inhibitor of the enzyme TS, a critical enzyme in the de novo synthesis of thymidylate. The formation and stabilization of a tight covalent ternary complex of TS-FdUMP-CH₂THF is dependent on and directly correlated with the presence and the concentration of the folate CH₂THF. The addition of LV, which forms an excess of CH₂THF, stabilizes the ternary complex and results in a marked reduction in the amount of TS available to catalyze the conversion of dUMP to dTMP, which is essential for normal DNA synthesis, thus enhancing cytotoxicity (11, 12). In lung cancer, using a panel of small cell lung cancer and NSCLC cell lines, we have previously demonstrated that the cytotoxicity of fluorinated pyrimidine can be enhanced by LV (21).

The antitumor activity of CDDP may be explained in part by DNA cross-linking (29, 30), interactions with the cell surface nucleic acids (31), or interactions with the plasma membrane (32, 33). Also, CDDP can inhibit methionine uptake into tumor cells and cause perturbation of the methionine pools (32, 33). Cells may respond by increasing methionine biosynthesis and increasing the pools of folate cofactors (changes in the methionine pools have been shown to alter the intracellular concentration of reduced folates) (34). When CDDP is ad-
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that LV has an independent supraadditive effect due to its deoxyfolate which is a product of expanded CH₂THF pools because of the TS-FdUMP-CH₂THF ternary complex may be responsible for the decrease the binding of FdUMP to TS. The resultant stabilization of the patients with NSCLC. Our results suggest that CDDP plus FUra have further clinical investigation in properly designed clinical trials in achieving clinical and pathologically confirmed complete responses in (9). More recently, continuous infusion of CDDP, FUra, and LV has been demonstrated since LV significantly improves the therapeutic effect of FUra without an increase in toxicity as compared with FUra alone (9). More recently, continuous infusion of CDDP, FUra, and LV has been shown to be a highly active chemotherapy regimen that can achieve clinical and pathologically confirmed complete responses in a substantial proportion of patients with advanced, local-regional squamous cell carcinoma of the head and neck (17-20) and early reports of phase II trials on patients with NSCLC have also shown encouraging results (35, 36). Because it appeared to have substantial activity in this in vitro study, a combination of CDDP, FUra, and LV deserves further clinical investigation in properly designed clinical trials in patients with NSCLC. Our results suggest that CDDP plus FUra have additive effects (and may be supraadditive at some effect levels) and that LV has an independent supraadditive effect due to its demonstrated enhancement of FUra activity.

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