Combination versus Single Agent Therapy in Effecting Complete Therapeutic Response in Human Bladder Cancer: Analysis of Cisplatin and/or 5-Fluorouracil in an in Vivo Survival Model

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ABSTRACT

An in vivo study of cisplatin (CDDP) and 5-fluorouracil (5FU) cytotoxicity was performed using a multidose matrix with a human bladder transitional cell carcinoma xenograft tumor line (DU4284) tested by subrenal capsule assay in 154 nude mice (NM-SRCA). Statistical analysis of initial growth inhibition at 20 days and host survival demonstrates therapeutic, cooperative interaction. Toxic doses of either CDDP or 5FU alone as well as low-dose combinations provided modest or no survival benefit. The single dose of CDDP (7 mg/kg) and of 5FU (100 mg/kg) was best (by analysis of efficacy and toxicity) of those tested and caused >97% initial regression. While 94% of controls incurred tumor deaths by 225 days, 75% treated at this dose were tumor free and likely cured. Our conclusions were: (a) NM-SRCA human xenograft testing is excellent for rapid in vivo screening of promising treatment strategies to evaluate for efficacy at acceptable toxicity, but confirmation of true therapeutic impact should be sought by correlating initial growth inhibition with host survival; (b) enhanced survival seen only when CDDP/5FU are used together (versus either single agent) supports the value of pursuing histotype-specific screening of potentially synergistic drug combinations; and (c) of clinical relevance, human transitional cell carcinoma is now identified as a histotype in which a therapeutic, cooperative interaction between CDDP/5FU has been demonstrated in vivo.

INTRODUCTION

Despite the continued development of clinically more effective, systemic, cytotoxic regimens such as the methotrexate/vinblastine/doxorubicin/cisplatin combination for the treatment of advanced TCC of the urothelium (bladder, ureter, and renal collecting system), cure from systemic therapy remains rare (1). While the search for new cytotoxic agents with enhanced efficacy for this tumor histotype remains critical, it is most important that further improvement in the use of presently available cytotoxic compounds be pursued. Of the numerous developmental, therapeutic strategies being investigated, the identification and exploitation of potentially synergistic drug combinations remain an important direction worthy of continued emphasis. The theoretical benefit of exploiting the concept of drug synergism, an enhanced biological response exceeding the “additive” effect of each single component, has been recognized for many years. Moreover, the putative value of combination drug therapy has gained general acceptance for a variety of theoretical reasons. However, most combination drug regimens now actively used clinically have been empirically derived (2). The combination of agents with single-agent activity, preferably with nonoverlapping toxicities, usually has been the rationale for the development of most such regimens (3).

The drug combination of CDDP and 5FU is considered potentially synergistic; cooperative, therapeutic interaction has been demonstrated (albeit not always by rigorous statistical analysis) in several histiotype of both human and marine malignancy (4–6). The biochemical basis for such an effect is incompletely understood; the ability of CDDP to modulate tetrahydrofolate availability to thymidine synthase is one mechanism which has been recognized (7). Because CDDP is commonly considered the most active single agent for the treatment of advanced urothelial carcinoma, it has been the cornerstone upon which combination drug regimens have been designed over the past decade. Despite single-agent activity of both CDDP and 5FU in bladder cancer (8), relatively little attention has been given specifically to evaluating the clinical utility of this drug combination for advanced bladder carcinoma. Moreover, no attention has been given to evaluate the potential for cooperative interaction of these drugs in a bladder cancer preclinical model.

An assessment of in vivo therapeutic response of cytotoxic agents directed against histiotype-specific human neoplasms is possible using nude mouse-supported human tumor xenografts evaluated after secondary implantation in test host animals. Using our adaptation (9) of the NM-SRCA originally described by Bogden et al. [Ref. 10; but not the subsequent, controversial assay later described by Bogden et al. (11)] using immunocompetent mice, we have successfully demonstrated the substantial chemosensitivity of the nude mouse-supported human bladder cancer xenograft tumor line (DU4284) to CDDP in this short-term growth inhibition assay (12); moreover, the ability of the chemosensitizer dipryridamole to potentiate the efficacy of CDDP and 5FU at acceptable host toxicity has been demonstrated in this intact, pharmacodynamic in vivo environment (13). While augmented cytotoxicity analyzed by the subrenal capsule short-term growth inhibition assay was seen when CDDP and 5FU were used together in that study, the design of that study was not amenable to an analysis of the degree of potentiation in the absence of a comprehensive, matrix dose approach. Furthermore, the true therapeutic impact on host survival of this drug combination in relation to the single agents was not assessed. Because no previous correlation of short-term growth inhibition with the NM-SRCA with host survival has ever been performed, the impact on host survival of such treatment approaches could not be predicted.

Herein is presented a comprehensive analysis of the impact of CDDP and 5FU on a human TCC xenograft which, when these two agents were combined, demonstrated a profound, therapeutic cooperativity in vivo capable of curing the host. This effect was seen only when these drugs were used in combination but not singly, even at high doses of the single agents. Correlation of the initial extent of response (judged by short-term growth inhibition) demonstrates that a profound growth inhibition must be achieved with a single treatment cycle in order to favorably translate into enhanced host survival. Only
when the drug combination was used could that extent of response be achieved at acceptable host toxicity.

**MATERIALS AND METHODS**

**Human Tumor Xenografts.** Tumor tissue was obtained from heterotransplants of human bladder TCC line DU4284 (Ref. 12; passage 19) which have been maintained subcutaneously by serial passage in nude mice. Nude mice (designated by Jackson Labs as BALB/c AnBomUrd) used in this study were produced from our inbred breeding colony and maintained in a true barrier facility at the Duke University Medical Center. Tumor tissue removed from mice was bathed in RPMI 1640 and implanted within 2–3 h.

**Subrenal Capsule Assay.** The NM-SRCA conceptually described by Bogden et al. (10) and modified by us (9) was used. The subrenal capsule as an implantation site is ideal because of the rapid tumor growth possible in a well-vascularized environment. Direct in situ measurement by stereoscopic zoom microscope (Olympus Model SZH with an ocular micrometer) was performed at ×11.4 such that 10 ocular units were equal to 1.1 mm. Tumor width and length were recorded in ocular units. All studies were performed along established, institutional animal welfare guidelines concordant with NIH species criteria. Female nude mice (8–12 weeks old) were used. The NM-SRCA protocol used was: day 1, tumor implantation; day 6, remeasurement; day 7, treatment; and day 20, final tumor measurement. Animals bearing tumors which failed to grow by the day-6 measurement were excluded; cohorts were randomly established from the pool of animals with actively growing tumor. Based on initial pilot studies of single-agent efficacy and toxicity ranges, a matrix design of combination doses as well as corresponding single-agent doses was established. Stock solutions were prepared using 0.9% sodium chloride to reconstitute clinical preparations of CDDP (Bristol-Myers) and 5FU (Quad). These were freshly prepared on each treatment day. All drugs were given i.p. on day 7. When both drugs were given, CDDP was always given first but followed immediately by 5FU for each animal (-2-min interval). The control cohort was treated with 0.9% saline given i.p. on day 7. A secondary control cohort was established for each single agent without surgery; on day 6 to be assured that this surgical procedure did not affect tumor growth or therapy; no effect was seen (data not shown). Doses of CDDP were 11, 7, and 3.5 mg/kg, and doses of 5FU were 150, 100, and 50 mg/kg. CDDP/5FU combinations of the above single-agent dosages were used as demonstrated in Table 1.

**Data Analysis of Growth Inhibition and Survival.** Growth ratios were calculated from estimated tumor volumes (calculated as LxW^2/2) determined by optical measurement on days 1, 6, and 20. The tumor volume ratios of both pretreatment (day 6/day 1) and posttreatment (day 20/day 6) growth were then determined. This latter ratio (day 20/day 6) during the experimental treatment time frame is designated as the tumor growth ratio. Mean tumor growth ratios of all cohorts were calculated. Statistical analyses were performed on the raw tumor volume data as well as these growth ratios. However, to enhance comparisons between the treatment cohorts for graphical depiction, extent of growth inhibition was normalized against the control cohort by calculating the RTS (or simply, % tumor survival) as:

\[
\text{% Tumor survival} = \frac{\text{Mean TGR}_{\text{exp}}}{\text{Mean TGR}_{\text{control}}} \times 100
\]

Using this mode of analysis, the quantitative level representing complete tumor growth inhibition is dependent on the degree of growth of the controls over the period of days 6–20.

Survival was assessed daily. The animal was considered agonal by virtue of minimal movement except when stimulated, and these animals were then euthanized humanely. A meta-analysis of the assessment of the impact of different treatment designs on survival was made at 80 days. At this point, the control group as well as all treatment cohorts except the highest-dose 5FU cohort and the high-dose combinations had achieved a median survival. To determine whether the host animal could be cured of disease by this drug combination, monitoring of these animals was continued; all dying or dead animals were necropsied to determine the presence of tumor. In the control animals, extremely large tumor masses were clearly evident. By 225 days, no animal had died of tumor for over 75 days; therefore, the study was terminated. All animals were necropsied except for three in the 7 mg/100 mg group. In these animals, fresh, explanted DU4284 was implanted in the undisturbed kidney. At day 245, these 3 animals were euthanized; necropsy demonstrated viable tumor growing rapidly in the second renal site but not in the original implantation site or elsewhere in the animal, indicating that resolution of the original tumor explants was not the result of an activated immune system in these nude mouse hosts. Of those animals surviving to the study termination, none had any evidence of remaining tumor despite the fact that all had documented tumor growth prior to treatment (Table 2).

**Statistical Analysis.** Due to the inherent nonnormality of the tumor volumes, nonparametric methods were used for statistical analysis. Tumor volume ratios (day 20/day 6) were analyzed to compare tumor growth between treatment groups. The Kruskal-Wallis test (14) was used to ensure that all cohorts were comparable at the inception of treatment. Analyses of these animals were carried out on day 7 (sample size of each cohort in parentheses).

**RESULTS**

**Impact of Cytotoxic Agents on Bladder Tumor Growth.** Table 1 illustrates the tumor volumes recorded on days 1, 6, and 20. Tumor volume growth ratios were also examined (Table 2) in order to adjust for any possible effect that pretreatment tumor size might have on tumor growth rate and measurement of tumor efficacy. While the changes in tumor volume ratios from days 1 to 6 were not statistically different (\( P = 0.834 \)), the growth from days 6 to 20 (posttreatment
growth ratios) differed significantly across treatment cohorts \((P < \) 0.0001). To analyze the amount of growth inhibition, the base 10 logarithms of the day 20:day 6 ratios were used. For the animals treated with CDDP compared to controls, there was a significant dose effect \((P = 0.0004)\). Likewise, there was a significant decrease in the day 20:day 6 tumor volume ratios for increasing doses of 5FU \((P = 0.0018)\).

Tumor growth inhibition was virtually complete \((97\% \text{ tumor inhibition})\) at the 7/100 dose, which is only 60% of the respective LD_{10} dosages for each agent. Full LD_{10} dose level combinations showed even more cytotoxic effect, but the differences due to the potency of the combination were not obvious from the tumor inhibition achieved with the 7/100 dose. This degree of efficacy \((>95\% \text{ tumor inhibition})\) is encountered at or slightly higher than 7 mg/kg of CDDP. A marked improvement of efficacy was noted in doubling the dose of both CDDP and 5FU between 3.5/50 and 7/100; moreover, an additional, substantial enhancement of efficacy over single-agent doses was seen.

Fig. 2 depicts RTS achieved with the various drug dose combinations in the study matrix organized by CDDP dosage. In reviewing the RTS response data depicted in Fig. 2, two echelons of response are evident, roughly defined by a RTS threshold of 10%. It is particularly noteworthy that dose escalation of 5FU had the most profound impact on tumor response, far more than escalation of CDDP. Whereas only a minimal growth inhibition was seen at 3.5/50, doubling the dose of 5FU improved the response almost by one log of response. In contrast, doubling the CDDP dose at the low-dose 5FU \((3.5/50 \text{ versus } 7/50)\) improved response at best by a factor of 2. At the high doses, total eradication of tumor occurred visually (by dissecting microscope) in some animals.

An analysis of the extent of therapeutic interaction was performed using regression analytic techniques for trend and association using the initial growth inhibition data of Tables 1 and 2. To test for drug interaction, only the results for animals receiving doses of CDDP <11 mg/kg and of 5FU <150 mg/kg were analyzed (including drug combinations and the untreated controls) in order to avoid transforming the large number of zero day-20 volumes \((i.e., \text{ no apparent tumor remaining visually})\). The results of this analysis are presented in Table 3. Variation in the doses of both CDDP and 5FU had a statistically significant impact on tumor inhibition. Moreover, this analysis demonstrated a statistically significant \((P = 0.006)\) “interaction” term, the departure from “additivity” in the regression model, for the CDDP/5FU combination consistent with the conceptual framework of cytotoxic synergism.

**Toxicity.** Substantial host toxicity was encountered whenever the maximum dose of 5FU \((150 \text{ mg/kg})\) was used in a combination dose. Similar toxicity was also seen at the medium 5FU \((100 \text{ mg/kg})\) dose.
when combined with the maximum dose of CDDP (11 mg/kg). Toxicity deaths all occurred within 10 days of treatment and at least 48 h after surgery. An analysis of the animal weights on days 6 and 20 was undertaken to relate toxicity to cytotoxicity. The relative weight loss levels considered acceptable before an inherent, calorie-restricted tumor inhibition is seen (20). All animals had regained their weight after surgery. An analysis of the animal weights on days 6 and 20 was comparable across groups. However, the high-dose cohorts did show marked weight loss. The greatest weight after surgery. An analysis of the animal weights on days 6 and 20 was the low-dose cohorts were comparable across groups. However, the efficacy was achieved with minimal host toxicity was the 7/100 drug restricted tumor inhibition is seen (20). All animals had regained their who died due to surgery are treated as censored events (19). At the termination of the study, 59% of the animals treated with the effective combination dose and higher were alive, while only 6% of the controls were alive. In those controls, extremely large tumor masses were evident. The one living control animal had, at necropsy, an atrophic, infarcted kidney, probably due to torsion of the renal pedicle (effectively, autonephrectomy) from inappropriate manipulation at the time of day-20 surgery. There were 2 additional deaths due to animal husbandry on day 222 immediately prior to study termination, which were considered cures since necropsy showed no tumor. Of those animals surviving to the termination of the study, none had any evidence of remaining tumor, despite the fact that all had documented tumor growth prior to treatment (Table 2).

Statistical analysis of survival was undertaken using analysis of Kaplan-Meier estimates (Table 5). Median survival with confidence intervals are also shown in Table 5 to allow comparison of drug doses and combinations. Probability of surviving 30 days was determined because this would primarily reflect toxicity. In contrast, the probability of surviving 90 days (as well as the median survival) would reflect both toxicity as well as tumor-related deaths. As delineated in Table 5, no median survival had been attained in four cohorts; these correspond to those cohorts having a marked initial growth inhibition of <10% RTS (Fig. 2). Moreover, in analyzing the 90-day survival probabilities, the 7/100 dose combination demonstrated the highest probability survival (0.83) when both efficacy as well as toxicity was factored into the assessment.

The proportional hazards regression of the full factorial experiment, which includes tumor-related, surgical, and toxic deaths, found an interaction of improved long-term survival, using all doses, for the combination of CDDP/5FU; however, it did not achieve statistical significance (P = 0.199). Refitting the model without the interaction term finds the effect of each drug on survival to be significant. Increased doses of CDDP reduced the risk of tumor related death

**Table 3 Test for trend and association of log relative tumor volumes**

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Standard Error</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDDP dose response regression</td>
<td>-7.19 X 10^{-2}</td>
<td>1.80 X 10^{-2}</td>
</tr>
<tr>
<td>5FU dose response regression</td>
<td>-5.43 X 10^{-3}</td>
<td>1.60 X 10^{-3}</td>
</tr>
<tr>
<td>CDDP &amp; 5FU interactiona</td>
<td>-1.34 X 10^{-3}</td>
<td>4.73 X 10^{-4}</td>
</tr>
</tbody>
</table>

a For each variable, the estimated regression coefficient (SE) and the two-sided P assessing statistical significance are shown.

b The "interaction" term is the departure from additivity in the regression model.

**Table 4 Deaths by treatment**

<table>
<thead>
<tr>
<th>CDDP</th>
<th>5FU</th>
<th>n</th>
<th>Total dead</th>
<th>Percentage</th>
<th>Nontumor deathsb</th>
<th>Tumor death</th>
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<tr>
<td>0</td>
<td>0</td>
<td>18</td>
<td>17</td>
<td>94</td>
<td>0</td>
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<td>7</td>
<td>7</td>
<td>100</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
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<td>10</td>
<td>10</td>
<td>100</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>150</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>89</td>
<td>0 (+1°)</td>
<td>7</td>
</tr>
<tr>
<td>3.5</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>100</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>50</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>100</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
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<td>7</td>
<td>7</td>
<td>64</td>
<td>0</td>
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</tr>
<tr>
<td>150</td>
<td>10</td>
<td>4</td>
<td>4</td>
<td>40</td>
<td>2</td>
<td>2</td>
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<tr>
<td>7</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>88</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>50</td>
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<td>9</td>
<td>82</td>
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</tr>
<tr>
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<td>12</td>
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<td>3</td>
<td>26°</td>
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<td>27</td>
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<tr>
<td>11</td>
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<tr>
<td>50</td>
<td>6</td>
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<td>4</td>
<td>67</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
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<td>7</td>
<td>7</td>
<td>78</td>
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<td>150</td>
<td>10</td>
<td>4</td>
<td>4</td>
<td>40</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

a All animals up to 225 days of observation.
b Toxicity deaths unless indicated.
c Anesthetic death at day 20 surgery.
d Does not include 2 husbandry deaths.

d **Impact of CDDP/5FU on Host Animal Survival.** Survival was monitored carefully. At 80 days, essentially all groups (except those having an initial RTS of 10% or less) had survival curves mirroring the control cohort, which had reached a 50% median survival. The crude survival data for the entire study by cause and treatment group are depicted in Table 4. There were eight early deaths which were attributable to drug toxicity. There were also two deaths on day 20 which resulted from the operative anesthesia to view and measure the tumor volumes. All other deaths were deemed tumor related. Animals who died due to surgery are treated as censored events (19). At the termination of the study, 59% of the animals treated with the effective combination dose and higher were alive, while only 6% of the controls were alive. In those controls, extremely large tumor masses were evident. The one living control animal had, at necropsy, an atrophic, infarcted kidney, probably due to torsion of the renal pedicle (effectively, autonephrectomy) from inappropriate manipulation at the time of day-20 surgery. There were 2 additional deaths due to animal husbandry on day 222 immediately prior to study termination, which were considered cures since necropsy showed no tumor. Of those animals surviving to the termination of the study, none had any evidence of remaining tumor, despite the fact that all had documented tumor growth prior to treatment (Table 2).

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**Table 5 Probability analysis of dose-dependent overall survival**

<table>
<thead>
<tr>
<th>CDDP</th>
<th>5FU</th>
<th>Probability of surviving 30 daysa</th>
<th>Probability of surviving 90 daysa</th>
<th>Estimated median (days)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.944 ± 0.054</td>
<td>0.167 ± 0.088</td>
<td>66 (59, 76)</td>
</tr>
<tr>
<td>50</td>
<td>1.000</td>
<td>0.286 ± 0.171</td>
<td>0.100 ± 0.095</td>
<td>55 (54, 79)</td>
</tr>
<tr>
<td>100</td>
<td>1.000</td>
<td>0.625 ± 0.171</td>
<td>0.75 ± 0.171</td>
<td>95 (57, 121)</td>
</tr>
<tr>
<td>3.5</td>
<td>1.000</td>
<td>0.250 ± 0.153</td>
<td>0.454 ± 0.150</td>
<td>67 (52, 72)</td>
</tr>
<tr>
<td>50</td>
<td>1.000</td>
<td>0.375 ± 0.171</td>
<td>0.700 ± 0.145</td>
<td>84 (77, 88)</td>
</tr>
<tr>
<td>100</td>
<td>1.000</td>
<td>0.833 ± 0.108</td>
<td>0.808 ± 0.122</td>
<td>No medianc</td>
</tr>
<tr>
<td>150</td>
<td>0.909</td>
<td>0.880 ± 0.139</td>
<td>0.700 ± 0.145</td>
<td>90 (81, 104)</td>
</tr>
</tbody>
</table>

a Estimates and SE from Kaplan-Meier estimates.
b Estimated median survival time and 95% confidence interval in parentheses.
c Deaths occurred in this group of animals.
d All animals in this group had died by this time.
e The data do not allow computation of upper limit to confidence interval.
f More than 50% of the animals were still alive at time of analysis (termination of study); therefore, the median was inestimable.
cohort. Note essentially no difference in survival of single-agent dosage from controls (7/100) compared to cohorts treated with a single agent at the same dose and was not different from controls. Early deaths in the combination cohort are toxicity deaths. dosage (11/150). Note some delay in progression but little or no enhanced difference in the hazard functions for the full data set suggests nonproportionality model without the animals receiving 11 mg/kg of CDDP or 150 mg/kg when used together (3.5/50) despite a modest, short-term growth combinations as well as the control cohort and the survival curve of expected if the two single-agent treatments were additive on this scale (P = 0.014). Figs. 3, 4 and 5 represent the Kaplan-Meier survival distributions for the low (3.5/50), middle (7/100), and high dose (11/150) drug combinations as well as the control cohort and the survival curve of the respective single agent. As one can see in Fig. 3, no enhanced survival is seen with low doses of CDDP alone, 5FU alone, or even when used together (3.5/50) despite a modest, short-term growth inhibition observed acutely. In marked contrast, Fig. 4 demonstrates the effect of doubling the dose of each drug used alone or together. While essentially no enhanced survival was seen with the 100 mg/kg 5FU dose alone and only a modest survival advantage was seen with the 7 mg/kg CDDP dose alone, 75% of the animals were cures at termination of the study when treated with these doses in combination. The high dose combination (11/150) also produced substantial survival benefit; however, host toxicity was marked (Fig. 5). It is of interest that the highest toxic dose of 5FU (150 mg/kg) did result in an RTS of 21% (Fig. 1); this corresponded to a median survival of 95 days, the longest median survival of any of the single-agent treatment cohorts. However, despite some delayed progression as a result of this high dose, the survival curve in Fig. 5 showed progressive deterioration and tumor-related death of nearly the whole treatment cohort, while the 7/100 combination cohort animals still surviving at 225 days showed no tumor, indicating probable cure. Finally, as one can see in Fig. 5, the several early deaths from toxicity diminished the overall survival of the cohort, despite what appeared to be, otherwise, a high cure rate. Consequently, the combination of 7 mg/kg of CDDP and 100 mg/kg of 5FU offers the best survival with the least toxicity in this study setting.

**DISCUSSION**

This study demonstrates that the combination of CDDP and 5FU is capable of profound in vivo tumoricidal efficacy at well-tolerated doses against a human TCC xenograft model in nude mice. This is the first comprehensive study in which a positive in vivo therapeutic interaction, defined by statistical analysis, between CDDP and 5FU has been demonstrated using a human epithelial neoplasm. This combination is capable of essentially achieving a “cure” after a single treatment cycle of this combination, a phenomenon not seen before by us using other drugs to treat human bladder cancer xenografts. Furthermore, while substantial, acute tumor inhibition could be achieved with single components of this combination alone, no substantial survival benefit was seen in those cohorts (compared to untreated controls), even when the single-agent dosage was at highly toxic levels. Only when the two drugs were used in combination using at least a moderate dose intensity did one see a substantive survival benefit. One of the rationales for clinical use of combination drug therapy is the potential benefit of drug synergy, a strategy strongly espoused by Wittes and Goldin (2). This study is a striking demonstration of the potential clinical efficacy of combination drug therapy when the components have a cooperative, therapeutic interaction suggesting synergism. Moreover, this is the first major study in which a correlation of acute tumor growth inhibition assessed by the NM-SRCA in nude mice has been made with survival. Our study demonstrates that substantial tumor reduction (greater than a 1 log reduction in tumor growth when compared to controls) needs to be achieved to have any survival benefit in such an animal model with a single dosage treatment schedule. This observation has an interesting parallel to clinical observations. It has been shown that only advanced bladder cancer patients who have achieved a very substantial, essentially complete response derive a survival benefit from their therapy; however, those partial responders (a 50% reduction of the index lesion) do not (21). Extent of response has never been correlated with host survival when tumor has been implanted at the subrenal capsule site; this correlation would now allow us the ability to interpret the results of a subrenal capsule assay (using human xenografted tissue in nude mice) with a greater appreciation of the degree of acute tumor growth inhibition likely necessary to change animal survival. Finally, and perhaps most importantly, these results suggest substantial value in reassessing a role for CDDP plus 5FU as a component in the treatment of advanced bladder cancer.

This *in vivo* study is unique in that, while the “synergistic” character of CDDP and 5FU has been accepted conceptually by many (primarily on the basis of *in vitro* data), demonstration of such augmented therapeutic interaction *in vivo* against epithelial neoplasms has been sketchy, and essentially no valid statistical analysis of such efficacy has been performed to confirm a therapeutic interaction. Many years ago, Schabel et al. (4) reported what they interpreted as synergy between CDDP and 5FU (but without accompanying statistical analysis); the tumor line studied was a murine leukemia cell line (L1210). We were concerned that such conclusions regarding putative thera-
peutic cooperativity of CDDP/5FU could not necessarily be translated to human epithelial neoplasms: (a) metabolic differences between murine and human tumors are well appreciated, and such differences could alter potentially synergistic drug relationships; (b) and perhaps more importantly, conclusions drawn from study of bone marrow-derived malignant cell lines may be inapplicable to epithelial neoplasms. For example, it is recognized that at least one biochemical mechanism for the synergy derived from the combination of CDDP and 5FU is the ability of CDDP to increase reduced folate within the cell (7). Tetrahydrofolate participates in the formation of stable ternary complexes with thymidylate synthase and FuUMP. However, the degree of potentiation achievable with folic acid (leucovorin) augmentation of 5FU cytotoxicity by increasing intracellular reduced folate appears to be cell-line dependent (leukemia versus epithelial cell lines). Degrees of potentiation exceeding 3-5 times (and as high as 20 times) the efficacy of the fluoropyrimidine used alone are commonly demonstrated when bone marrow stem-cell-derived malignant lines are studied; however, degrees of potentiation exceeding 2 times the effect of the fluoropyrimidine alone are rarely seen against epithelial cell lines (22). This would suggest that there may be metabolic differences critical to CDDP and/or 5FU sensitivity between bone marrow-derived and epithelial neoplasm. Consequently, demonstration of a cooperative, therapeutic effect of CDDP/5FU in vivo against epithelial neoplasms is important and clearly cannot be assumed. Little work evaluating this question comprehensively has thus far been reported. It is therefore significant that our study clearly shows statistically that a strong therapeutic interaction is achievable in vivo against this bladder neoplasm using both acute tumor growth inhibition and, perhaps more importantly, survival for analysis.

The in vivo chemosensitivity model that we used is an excellent one for the study of drug combinations for potential therapeutic merit; it is a rapid assay treating histiotypically stable tumors in a well-vascularized environment at the animal’s core temperature. We describe a modification of the technique originally described by Bogden et al. (10) using nude mice [not the largely discredited 6-day assay using immunocompetent mice later described by Bogden et al. (11)]. The 6-day subrenal capsule assay using immunocompetent mice has been abandoned for drug testing by most; it has subsequently been shown that substantial and unpredictable host lymphocytic infiltration can occur as early as 4 days, which can interfere with true assessment of cytotoxic response (23, 24). In our hands, xenografted tumors grew in nude mice without evidence of host-mediated cellular response; histological analysis of a large tumor burden of DU4284 even at 80 days (in a nearly agonal animal) fails to demonstrate any infiltration by lymphocytes. Moreover, use of survival models established by i.p. injection of tumor cell lines are of questionable value if the test drugs are to be administered i.p., potentially delivering an artifactual, extremely high, local drug concentration. In contrast, use of s.c. tumor implantation is also problematic because growth rates can be highly variable from animal to animal at this peripheral site, thereby resulting in statistical difficulties. While in vitro assays provide rapid information regarding cytotoxicity at relatively low cost, their usefulness is limited in assessing the comprehensive impact of drugs in combination with the therapeutic index, a concept incorporating the relative pharmacological efficacy at a given, limiting toxicity. Therapies that might show enhanced therapeutic efficacy in vitro also have the potential for markedly enhanced toxicity. For instance, our assessment of the combination of tumor necrosis factor and actinomycin D is a demonstration of such unrecognized “toxic synergism”; while others had shown substantial, augmented cytotoxicity of the two agents in combination, our study demonstrated marked lethal toxicity even at highly tolerated single-agent doses (25). Similarly, we have recently demonstrated that dipyridamole is a chemosensitizer capable of augmenting the cytotoxic efficacy of platinating agents (both CDDP and carboplatin). However, it was only by rigorous analysis of the “therapeutic index” in vivo that we recognized that marked enhancement of the therapeutic index is achieved with CDDP but not carboplatin (26); the enhanced efficacy attained using dipyridamole with the latter was offset by enhanced toxicity. Only by evaluating such drug combinations in vivo can one assess the true impact on the therapeutic index.

This study has demonstrated that, in our nude mouse/human xenograft model of human bladder cancer, CDDP/5FU in combination is capable of achieving a cure at highly acceptable levels of toxicity. The rate of survival of the animals treated with adequate doses of CDDP/5FU is in contrast to the single-agent efficacy of each component. While single-agent dosages escalated up to toxic levels did show acute tumor inhibition (but not to the same extent of response as the CDDP/5FU combinations), little or no survival benefit was seen. 5FU at the highest dose (Fig. 5) did induce a modest delay in death in some animals in the treatment cohort, but no plateau effect in survival was seen to suggest cure in any substantial proportion of the animals. Low doses of 5FU and CDDP (33 and 51% of LD10 dosage, respectively) were ineffective as single agents in the 20-day NM-SRCA and were only mildly cytotoxic in combination; however, no survival advantage of combined 5FU and CDDP at low dose was observed. Striking tumor growth inhibition translating into enhanced survival was only seen with any combination using a dose of CDDP >3.5 mg/kg and of 5FU >50 mg/kg, suggesting the necessity that threshold intratumoral drug concentrations (particularly 5FU) must be achieved for cell death to occur.

5FU is generally considered to have single-agent activity for TCC, but this is based primarily on old clinical trials (27). However, the use of CDDP plus 5FU has received little attention for this tumor, probably because of the popularity of CDDP/methotrexate-based combinations such as methotrexate/vinblastine/doxorubicin/cisplatin and cisplatin/methotrexate/vinblastine. The CDDP/5FU combination has been investigated in only one clinical trial (combined with doxorubicin); partial responses were seen in 46% of patients (28). However, undoubtedly necessitated by the added myelosuppressive effect of the doxorubicin, it should be noted that the dosage of 5FU in that study was lower than that commonly used when 5FU is used in combination with only CDDP. Our work presented here demonstrates that, when compared to the results with “full dose” (7/100 CDDP/5FU, use of only “half-dose” 5FU (50 mg/kg) even with “full dose” CDDP results in substantially less (15-fold) tumor growth inhibition (Table 2) with less likelihood of surviving 90 days (Table 5) and shorter, actual survival (Table 4; 18 versus 75%, respectively). In contrast, the CDDP/5FU combination has received considerable attention in colon and head and neck cancers. When 5FU is administered as an i.v. infusion over several days, this combination is capable of substantial efficacy (60-70% total response rate) in head and neck cancers (29, 30). The potential of such a cytotoxic combination for the treatment of advanced bladder cancer, perhaps further augmented with chemosensitizers such as dipyridamole or potentiators such as leucovorin, may be worth careful assessment clinically.

REFERENCES


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Combination \textit{versus} Single Agent Therapy in Effecting Complete Therapeutic Response in Human Bladder Cancer: Analysis of Cisplatin and/or 5-Fluorouracil in an \textit{in Vivo} Survival Model


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