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Bromodeoxyuridine improves the cytotoxic effect of cisplatin: a comparison with 5-fluorouracil

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Abstract We compared the effects of the radiosensitizers, 5-bromo-2′-deoxyuridine (BUdR) and 5-fluorouracil (5-FU) alone and in combination and *cis*-diamminedichloroplatinum (cisplatin, DDP) on the growth of Bl6 amelanotic melanoma (Bl6a) tumors in mice. In a preliminary study, tumor growth was significantly inhibited in the presence of BUdR and was further reduced with the combination of BudR and DDP. In a second experiment, BUdR was found to be more effective than 5-FU when used in combination with DDP. At the completion of the study, tumor volumes as a percentage of control values in mice treated with a single drug were as follows: 5-FU (50 mg/kg per day for 7 days) 76.5% ($P < 0.05$), BUdR (100 mg/kg per day for 7 days) 68% ($P < 0.05$) and DDP (5 mg/kg × 3) 54% ($P < 0.01$). Combining 5-FU and DDP at these dosages reduced volumes to 38% ($P < 0.01$), while BUdR + DDP-treated mice had tumor volumes only 28% ($P < 0.001$) the size of untreated controls. Furthermore, the toxicity, as demonstrated by a decrease in body weight and an increase in mortality, was more severe in mice receiving 5-FU than in those receiving in BUdR. DDP interacts synergistically with either BUdR or 5-FU in its cytotoxic action in vivo. No such relationship could be demonstrated in vitro, suggesting that the pharmacologic activity of these drugs may be responsible for the antitumor activity rather than direct cytotoxic effects. We propose that BUdR is more effective than 5-FU as a potentiator of DDP in this murine melanoma model.

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Introduction

Cis-diamminedichloroplatinum (DDP) is used as the treatment of choice in a number of human malignancies. The mechanism of action is related to the formation of DNA adducts, including DNA–protein crosslinks, DNA monoadducts, lethal intrastrand and interstrand DNA crosslinks [3], as well as interactions with the plasma membrane [24]. A major problem limiting the clinical effectiveness of DDP is the ability of tumor cells to acquire resistance by a variety of mechanisms [2, 4, 5, 9, 11, 14, 20]. One strategy used to overcome resistance is to combine DDP with other chemotherapeutic agents, including the halopyrimidine, 5-fluorouracil (5-FU). Nevertheless, certain tumors become refractory to the combination of 5-FU and DDP with prolonged treatment, and at present, there is no clinical backup available. We explored the use of the halogenated pyrimidine, 5-bromo-2′-deoxyuridine (BUdR), in combination with DDP, and compared its action with that of 5-FU, an agent known to act synergistically with DDP.

Prior in vitro studies had demonstrated activity of certain halogenated pyrimidines as chemosensitizers. Russo et al. [22], for example, showed that 5-iodo-2′-deoxyuridine markedly potentiates the cytotoxic action of DDP and bleomycin against tumor cells. BUdR, which is structurally similar to 5-iodo-2′-deoxyuridine, has been tested as a radiosensitizer in a number of laboratory and clinical settings [1, 7, 12, 15, 26, 27]; however, no in vivo studies have been carried out using BUdR as a potentiator of chemotherapeutic agents.

It has been previously shown that promising in vitro strategies may fail at the complex tumor level [17]. Therefore it was imperative to study the relative activity of the halogenated pyrimidines in an animal tumor model. The murine B16a cell line, made partially resistant to DDP by repeated exposure to the drug, was

selected to evaluate the efficacy of BUdR as a potentiator in vivo.

Materials and methods

B16a tumor line

The B16a cell line was obtained from the Division of Cancer Treatment (DCT), National Cancer Institute, Tumor Repository (Frederick, Md.).

Mice

B16a tumors were propagated in male, syngeneic host mice (C57BL/6J) obtained from the Jackson Laboratory (Bar Harbor, Me.). Mice at 6–8 weeks of age, weighing 20–22 g and housed under identical conditions, with access to food and water ad libitum, were used in the experiments. In experiment 1, there were four groups (control, DDP, BUdR and DDP + BUdR) of 10–12 mice each. In experiment 2, an additional two groups (5-FU and DDP + 5-FU) were also tested. Mice were anesthetized with sodium pentobarbital (65 mg/kg) for osmotic pump implantation for delivery of either BUdR or 5-FU. All protocols and procedures were approved by the Providence Hospital Animal Care and Use Committee (Institutional Animal Care and Use Committee) and were consistent with the US Public Health Service and National Institutes of Health policies and guidelines.

Chemotherapeutic agents

A 10-mg vial of DDP (Platinol, Bristol Laboratories, Evansville, Ind.) containing 100 mg mannitol and 90 mg sodium chloride was reconstituted with 10 ml sterile water. Mice received 5 mg/kg body weight DDP intraperitoneally (i.p.) three times during the course of the study. In experiment 1, DDP was injected on days 6, 8 and 13 following osmotic pump implantation, and in experiment 2, DDP was administered on days 5, 8 and 12 after implantation of the minipumps. Control mice received a comparable volume of solution containing 10 mg/ml mannitol and 9 mg/ml sodium chloride.

In experiment 1, BUdR (Sigma Chemical Co., St. Louis, Mo.) was dissolved in a 0.1 *M* sodium bicarbonate buffer, pH 9.8, at a concentration of 200 mg/ml. The solution was loaded into Alzet model 2001 osmotic pumps (Alza Corporation, Palo Alto, Calif.) and then implanted subcutaneously into each mouse. These 200-µl osmotic pumps delivered 250 mg/kg per day BUdR at a rate of 1 µl/h over the course of 1 week. An osmotic pump containing bicarbonate buffer was implanted into each control mouse.

In experiment 2, the smaller Alzet micro-osmotic pump (model 1007D), which has a 100-µl capacity and pumps at the rate of 0.5 µl/h for 7 days, was utilized. These osmotic pumps were filled with BUdR, 5-FU, or buffer. BUdR (140 mg/ml) was dissolved in sodium bicarbonate buffer, loaded into pumps, and implanted subcutaneously into appropriate groups. These mice received 100 mg/kg per day BUdR. 5-FU (Sigma Chemical Co.) was suspended at a concentration of 70 mg/ml in sterile distilled water, the pH was increased to 11.0 with NaOH, and heated to dissolve. Pumps filled with this solution delivered 50 mg/kg per day. Control mice were implanted with a pump containing only buffer.

Development of DDP-resistant tumor line

Resistance to DDP was developed by sequential treatment of mice and B16a cells in culture with this drug. The procedure was modeled on the method of Onoda et al. [19]. Mice carrying Bl6a tumors received 5 mg/kg DDP i.p. at 1 and 2 weeks following tumor cell transplantation. After 3 weeks the primary tumor was harvested and minced, the cells were placed in culture and exposed to increasing doses (0.5–4.0 µ*M*, final concentrations) of DDP. These cells were then harvested, checked for viability, and reinjected into a new host mouse. This cycle was repeated two more times in order to increase DDP resistance. Cells surviving the final 4 µ*M* DDP treatment were used for the studies.

Study Protocol

In the first experiment, 48 mice were injected subcutaneously in the right flank with 0.35×10^6 melanoma cells. An osmotic pump was implanted subcutaneously into the left flank of each mouse 11 days later (day 0), when the tumor volumes had reached 30–40 mm^3. Of these pumps, 24 contained 0.1 *M* sodium bicarbonate buffer, and the other 24 contained BUdR (200 mg/ml), which was delivered at 250 mg/kg per day to each mouse over the course of 1 week. On days 6, 8 and 13 after osmotic pump implantation, one half of each group of mice received DDP (5.0 mg/kg, i.p.), while the other half was injected with the saline/mannitol control solution.

In the second experiment, two additional groups of mice were treated with 5-FU alone and the combination of DDP + 5-FU. A total of 80 mice were injected subcutaneously with 0.48×10^5 melanoma cells each. When tumor growth was evident 15 days later, micro-osmotic pumps were implanted subcutaneously in all 80 animals. Mice were divided into the following groups: control, BUdR (100 mg/kg per day) and 5-FU (50 mg/kg per day). One-half of each group received DDP (5.0 mg/kg, i.p.) on days 5, 8 and 12 after osmotic pump implantation, while the other half was injected with saline/mannitol.

Tumors were measured two or three times weekly using a sliding caliper, and the volumes were calculated using the formula $V = \pi/6 \times LW^2$, where W is the center of the tumor measured perpendicular to L [19]. The tumor volumes, normalized to 100 g body weight, were then calculated for each mouse.

On day 21 of experiment 1 and day 19 of experiment 2, all mice were euthanized, the tumors were excised and weighed, and the lungs were removed and placed in Bouin's fixative for later quantitation of superficial metastatic nodules.

In vitro studies

The cytotoxic effects of DDP plus either BUdR or 5-FU on cisplatin-resistant B16a cells (2500 cells/ml) in 96-well microtiter plates were compared. Cells (100 µl/well), were treated 24 h after plating with DDP alone (0.1–1.6 µg/ml, five dilutions), 5-FU alone (0.025–0.4 µg/ml, five dilutions) or BUdR alone (15.6–250 µg/ml, five dilutions) or combinations of DDP plus 5-FU or BUdR. Cells were further incubated for 3, 4 and 5 days and cell viability was quantitated using the colorimetric MTT (3,4,5-dimethylthiazol-2,5-diphenyl-tetrazolium bromide, Sigma Chemical Co.) assay of Mossman [18] with some modifications. Drug interactions were assessed by comparing the actual survival rate (SR) as a percent of control with that expected. The expected survival rate (E-SR) was calculated using the formula: E-SR = (SR by DDP alone) × (SR by 5-FU or BUdR alone)/100 [13]. A SR less than the calculated E-SR would indicate synergism. The cytotoxic effect of DDP on B16a cells made partially resistant to this drug and on the parent cell line were also compared.

Statistical analysis

The data were analyzed by multvariate analysis of variance for repeated measures and post hoc analysis with Duncan's multiple-range test. $P < 0.05$ was considered statistically significant for all comparisons.

Results

Two studies were carried out to examine the efficacy of BUdR as a potentiating agent on tumor cells partially

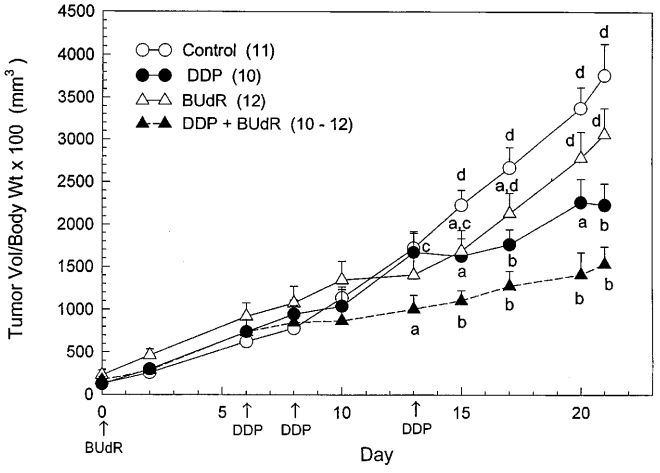

Fig. 1 Tumor volume/body weight (× 100) during the course of experiment 1. On day 0, osmotic pumps delivering 250 mg/kg per day (BUdR) were implanted subcutaneously. On days 6, 8, and 13 DDP (5 mg/kg) was injected intraperitoneally (*a* $P < 0.05$ compared with control group, *b* $P < 0.01$ compared with control group, *c* $P < 0.05$ compared with DDP + BUdR group *d* $P < 0.01$ compared with DDP + BUdR group). Numbers in brackets are the number of animals per group

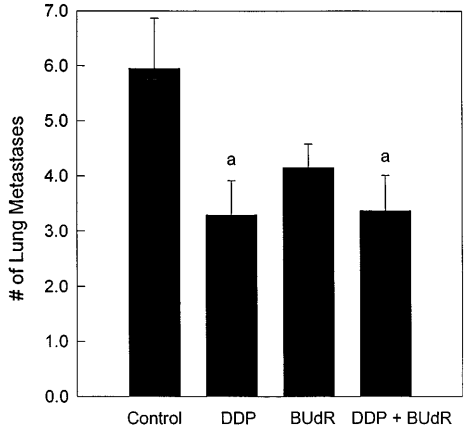

Fig. 2 Number of lung metastases in experiment 1. (*a* $P < 0.05$ compared with control group)

resistant to DDP. In the first experiment, it was evident from day 13 (Fig. 1) that tumor-bearing mice receiving the combination of DDP and BUdR had significantly smaller tumors than mice in the control or BudR groups ($P < 0.05$). By the end of the experiment, tumor volumes per 100 g body weight in control mice were 3764 ± 369 mm^3 (mean ± SE), while in mice treated with BUdR alone they were 3066 ± 311 mm^3 or 81% of controls, and 2233 ± 250 mm^3 or 59% of control volumes in animals injected with DDP alone ($P < 0.01$). In mice receiving the combination of BUdR and DDP, tumor volumes were reduced to 1534 ± 206 mm^3 or 41% ($P < 0.01$) of control values and were also significantly smaller than those in the BUdR group ($P < 0.01$).

Body weights were recorded in order to assess the toxicity resulting from the various treatment regimens. The BUdR and the BUdR + DDP groups showed significant weight loss ($P < 0.01$) from day 8 until the end of the study. The DDP group showed a decline ($P < 0.05$) in body weight starting from day 13 (data not shown). By the end of the study (Table 1) body weights of mice receiving DDP, BUdR, and DDP + BUdR had been reduced to 88.5%, 78.7%, and 69.6% of control values, respectively. Gross tumor weights determined at necropsy (Table 1) correlated well with the volumes calculated for this time-point. At the end of the study, mice that had received DDP + BUdR had significantly smaller tumors than those that had received either DDP ($P < 0.05$) or BUdR ($P < 0.01$) alone. Mice that had received DDP or DDP + BUdR had significantly smaller tumors ($P < 0.01$) and fewer metastatic lung colonies ($P < 0.05$) than the controls (Fig. 2). Although there was a reduction in the number of metastatic nodules in mice receiving BUdR alone, it was not statistically different from the control group.

A second experiment was then carried out to compare the effects of BUdR and 5-FU given alone and in combination with DDP on Bl6a tumor growth. This study was altered after learning from the first study that mice tended to dehydrate with drug treatment. All mice were given 1.5 ml lactated Ringer's solution (Baxter Healthcare Corp., Deerfield, Ill.) subcutaneously after each tumor measurement. As a result of these injections, body weights in the experimental groups did not fall below 79% of controls at any point during the study. However, mice that had received DDP alone or DDP in any combination regimen still had lower body weights than control animals ($P < 0.01$) by the completion of the study (Table 2).

Groups that were infused with 5-FU had the greatest mortality. By day 20, 3 out of 12 mice in the 5-FU

Table 1 Body weights and tumor weight/body weight at the end of experiment 1

Group	Body wt (g) ± SE	% of control	Tumor wt/body wt (× 100) ± SE	% of control
Control	29.6 ± 1.05[*4]	100	12.33 ± 0.93[*4]	100
DDP	26.2 ± 1.54[*1,4]	88.5	7.64 ± 0.88[*2,3]	62.0
BUdR	23.3 ± 0.84[*2]	78.7	10.29 ± 0.92[*4]	83.4
DDP + BUdR	20.6 ± 1.05[*2]	69.6	5.42 ± 1.06[*2]	44.0

[*1] $P < 0.05$ vs control group, [*2] $P < 0.01$ vs control group, [*3] $P < 0.05$ vs DDP + BudR group, [*4] $P < 0.01$ vs DDP + BUdR group

Table 2 Body weights and tumor weight/body weight at the completion of experiment 2

Group	Body wt (g) ± SE	% of control	Tumor wt/body wt (× 100) ± SE	% of control
Control	25.8 ± 1.17[*4]	100	8.25 ± 1.18[*4]	100
DDP	21.6 ± 1.18[*2]	83.7	5.49 ± 1.02[*3]	66.5
5-FU	23.8 ± 1.51	92.2	8.13 ± 1.62[*4]	98.5
BUdR	24.4 ± 0.72	94.6	5.37 ± 1.05[*3]	65.1
DDP + 5-FU	20.6 ± 1.31[*2]	79.8	4.25 ± 1.05[*1]	51.5
DDP + BUdR	21.5 ± 0.50[*2]	83.3	2.41 ± 0.29[*2]	29.2

[*1] $P < 0.05$ vs control group, [*2] $P < 0.01$ vs control group, [*3] $P < 0.05$ vs DDP + BUdR group, [*4] $P < 0.01$ vs DDP + BUdR group

groups, and 6 out of 13 in the DDP + 5-FU group had expired. No control animals died; however, one in each of the other three groups died, although no mortalities had been anticipated with the dosages selected for any of these chemotherapeutic agents.

The DDP + BUdR mice showed the greatest reduction in tumor volumes (Fig. 3). This difference was evident starting from day 12 after BUdR pump implantation. At this time mice treated with DDP plus BUdR had tumor volumes significantly smaller ($P < 0.05$) than the control and 5-FU groups. By the end of this second study (day 19), tumor volumes in the DDP (1103 ± 135 mm^3), DDP + 5-FU (784 ± 153 mm^3), and DDP + BUdR (577 ± 66 mm^3) groups were all significantly smaller ($P < 0.01$) than the untreated controls (2056 ± 279 mm^3). BUdR treatment alone also resulted in smaller tumors (1397 ± 242 mm^3) compared with control mice ($P < 0.05$). The tumors in mice that had received either 5-FU (1573 ± 332 mm^3) or BUdR alone were significantly larger than those in mice that had received a combination of BUdR and DDP.

The gross tumor weights per 100 g body weight (Table 2) in mice receiving both DDP and BUdR were significantly lower than in those receiving DDP, BUdR ($P < 0.05$) or 5-FU ($P < 0.01$) alone. The number of metastatic lung colonies (Fig. 4) was generally decreased in the BUdR, DDP + BUdR, and DDP + 5-FU groups. However, only the BUdR and BUdR + DDP groups were statistically different ($P < 0.05$) from the control group in this experiment.

In vitro studies showed that all three drugs have direct inhibitory effects on the growth of B16a cells. The parent cell line (not previously exposed to DDP) was significantly more sensitive to DDP than the partially resistant cells. For example, at a dose of 0.1 μg/ml the parent line had only a 61.5% survival rate compared with 84.9% in the partially resistant line. Effects of combinations of DDP plus BUdR or 5-FU were not synergistic (results not shown). For our in vivo studies, tumor weights calculated as a percent of the control at the end of the study were analyzed in a similar manner. The combinations of DDP plus BUdR or 5-FU were both highly synergistic. For experiment 1 a mean tumor weight of less than 51.7% of control for the DDP + BUdR combination was considered synergistic. The mean tumor weight of this group was 42.2% of the control. In experiment 2 an average tumor weight of less than 65.5% or 43.3% of control values would indicate synergism for the combinations of DDP and 5-FU or BUdR, respectively. Values of 51.5% (DDP + 5-FU) and 29.2% (DDP + BUdR) were obtained in this study.

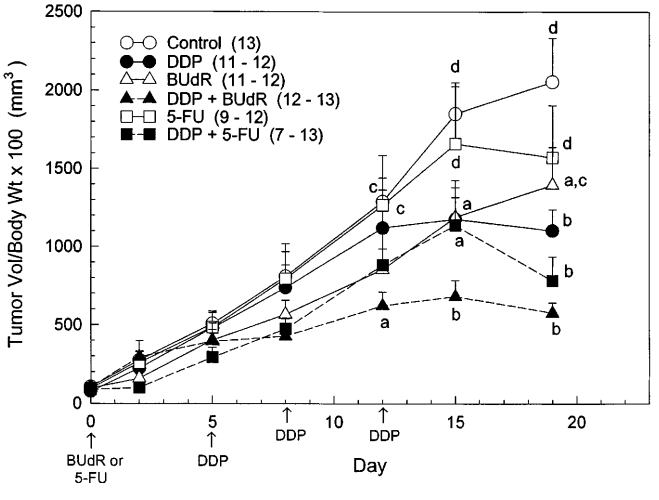

Fig. 3 Tumor volume/body weight (× 100) during the course of experiment 2. On day 0, micro-osmotic pumps delivering 100 mg/kg per day BUdR or 50 mg/kg per day 5-FU were implanted subcutaneously. On days 5, 8, and 12 after pump implantation, DDP (5 mg/kg) was injected intraperitoneally See Fig. 1 legend for further details

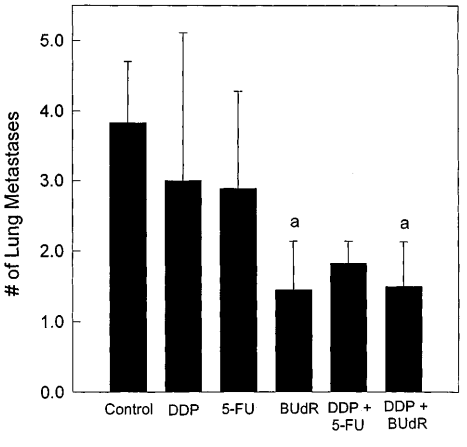

Fig. 4 Number of lung metastases in experiment 2 (*a* $P < 0.05$ compared with control group)

Discussion

The radiosensitizing capability of halogenated pyrimidines has been known for decades [8, 10]. Two such compounds, BUdR and 5-iodo-2′-deoxyuridine, have been tested as radiosensitizers in a number of laboratory [12, 16] and clinical studies [15, 26]. Another pyrimidine analog, 5-FU, has been successfully tested and used in the treatment of DDP-resistant tumor cells. We explored the possibility that BUdR could be used as a potentiator of DDP. Although the B16a cells were not made completely resistant to DDP in our study, BUdR did improve the cytotoxic effect of DDP.

In a second experiment, we compared BUdR and 5-FU in combination with DDP for their antitumor activities. Although both drugs did potentiate the cytotoxicity of DDP, the DDP + BUdR combination tended to give a greater reduction in tumor volume at a dose which was much less toxic to the mice.

Along with the reduced tumor sizes following drug treatment, it was also noted that the number of metastatic lung colonies was significantly reduced in the experimental groups compared with the control group. The DDP + BUdR group consistently had the fewest metastatic lung nodules. Possibly the smaller tumor load resulted in a decrease in tumor cell metastases. Whether these drugs exerted any effect on the metastasizing potential of tumor cells needs further investigation.

The dose of DDP selected (5 mg/kg) was equivalent to 0.42 mg/kg for a human or 16.8 mg/m^2. This compares well with that of DDP given in combination with 5-FU in clinical regimens. To convert from a mouse dose in terms of mg/kg to an equivalent surface area dose in terms of mg/kg in humans, a conversion factor of 1/12 is used. This result can then be multiplied by 40 in order to obtain a per square meter dosage [6]. The 250 mg/kg per day dose of BUdR was equivalent to 20.8 mg/kg per day or 832 mg/m^2 per day. Phillips et al. [21] used a similar dose of BUdR (800 mg/m^2 per day) as a sensitizing agent for radiotherapeutic treatment. BUdR was effective as a potentiator of DDP even when used at a much lower dosage (100 mg/kg per day) in the second experiment. 5-FU was delivered at a dose of 50 mg/kg per day. This would translate to an equivalent dose of 4.17 mg/kg per day or 167 mg/m^2 per day for a human patient. This dose is comparable to that used by Shimoyama et al. [25], who determined 50 mg/kg i.p. to be the maximum tolerated dose in their nude mouse model. Saikawa et al. [23] used 5-FU at doses ranging from 150 to 180 mg/kg i.p. in mice, which resulted in a rather high mortality. Tumor volumes in the 5-FU + DDP group were not reduced to the same degree as in the BUdR + DDP group with the dosage levels selected. Mice treated with both 5-FU and DDP also had the lowest body weights and the highest mortality. Clearly, the 5-FU + DDP combination was much more toxic to mice than the BUdR + DDP combination.

The mechanism by which 5-FU sensitizes cells to DDP has been studied [24]. The synergy between these two drugs may be due to their interaction at the folate level. DDP's interaction with the plasma membrane causes an inhibition of methionine uptake into the cell. As a result, the intracellular synthesis of methionine and folate cofactors increases. The folate cofactor (5,10-methylenetetrahydrofolate) is necessary for the tight binding of the major 5-FU metabolite, 5-fluorodeoxyuridine monophosphate (FdUMP) to dTMP synthase. Scanlon et al. [24] found that increased intracellular levels of the reduced folate caused a 2.5-fold increase in binding of FdUMP to dTMP synthase. DNA synthesis is thus inhibited by the depletion of dTMP in the cell. BUdR, on the other hand, incorporates into DNA in place of thymidine in actively proliferating cells during S-phase. As a result, BUdR may render the DNA more vulnerable to adduct formation or damage induced by DDP. The kinetics of interactions between BUdR and DDP need further investigation.

Our results suggest that it is the pharmacologic activity and interactions of these drugs in mice, rather than their direct cytotoxic effects, that is responsible for their antitumor activity. Both 5-FU and BUdR, which are cytotoxic when used alone, synergistically potentiated the cytotoxic effects of DDP in vivo but not in vitro.

In summary, the halopyrimidine BUdR improved the cytotoxic activity of DDP in this murine melanoma model. Whether the increase in cytotoxicity was a direct result of the lethal effect of BUdR on these cells, or whether BUdR actually sensitized them to DDP, needs further study. However, BUdR's cytotoxic activity compared well with that of 5-FU, an agent known to enhance the cytotoxic activity of DDP in DDP-resistant tumor cells. These preliminary studies suggest that BUdR may have a role as a primary therapeutic agent in the clinical setting, or in the treatment of tumors which have become resistant to DDP.

References

1. Al-Sarraf M (1989) Clinical trials with fluorinated pyrimidines in patients with head and neck cancer. Cancer Invest 7: 71
2. Bernal S, Speak J, Boeheim J, Dreyfuss A, Wright J, Teicher B, Rosowsky A, Tsao S, Wong Y (1990) Reduced membrane protein associated with resistance of human squamous carcinoma cells to methotrexate and cis-platinum. Mol Cell Biochem 95: 61
3. Chu G (1994) Cellular responses to cisplatin. J Biol Chem 269: 787
4. Chu G, Chang E (1988) Xeroderma pigmentosum group E cells lack a nuclear factor that binds to damaged DNA. Science 242: 564
5. Chu G, Chang E (1990) Cisplatin resistant cells express increased levels of a factor that recognizes damaged DNA. Proc Natl Acad Sci USA 87: 3324
6. Devita VT Jr (1993) Principles of chemotherapy. In: Devita VT Jr., Hellman S, Rosenberg SA (eds) Cancer, principles & practice of oncology. J. B. Lippincoft Company, Philadelphia, p 276

7. Dewey WC, Humphrey RM (1965) Increase in radiosensitivity to ionizing radiation related to replacement of thymidine in mammalian cells with 5-bromodeoxyuridine. Radiat Res 23: 538
8. Djordjevic B, Szbalski W (1960) lncorporation of 5-bromo- and 5-iodo-deoxyuridine into the deoxyribonucleic acid of human cells and effect on radiation sensitivity. J Exp Med 1 1 2: 509
9. Eastman A, Schulte N (1988) Enhanced DNA repair as a mechanism of resistance to cis-diamminedichloroplatinum. Biochemistry 27: 4730
10. Erickson RL, Szybalski W (1963) Radiation sensitizing properties of 5-iododeoxyuridine. Cancer Res 23: 122
11. Godvvin A, Meister A, O'Dwyer P, Huang C, Hamilton T, Anderson M (1992) High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis. Proc Natl Acad Sci USA 89: 3070
12. Goz B (1978) The effects of incorporation of 5-halogenated deoxyuridines into the DNA of eukaryotic cells. Pharm Rev 29: 249
13. Kase S, Kubota T, Watanabe M, Furukawa T, Tanino H, Ishibiki K, Teramoto T, Kitajima M (1993) Interferon beta increases antitumor activity of 5-fluorouracil against human colon carcinoma cells in vitro and in vivo. Anticancer Res 13: 369–374
14. Kawai K, Kamatani N, Georges E, Ling V (1990) Identification of a membrane glycoprotein overexposed in murine lymphoma sublines resistant to cis-diamminedichloroplatinum. J Biol Chem 265: 13137
15. Kinsella T, Mitchell J, Russo A, Morstyn G, Glatstein E (1984) The use of halogenated thymidine analogues as clinical radiosensitizers: rationale, current status, and future prospects. Int J Radiat Oncol Biol Phys 10: 1399
16. McLaughlin P, Mancini W, Stetson P, Greenberg H, Nguyen N, Seabury H, Heidorn D, Lawrence T (1993) Halogenated pyrimidine sensitization of low dose rate irradiation in human malignant glioma. lnt J Radiat Oncol Biol Phys 26: 637
17. McLaughlin PW, Lawrence TS, Seaburg H, Nguye, N, Stetson P, Greenberg HS, Mancini W (1994) Bromodeoxyuridine-mediated radiosensitization in human glioma: the effect of concentration, duration and fluoropyrimidine modulation. lnt J Radiat Oncol Biol Phys 30: 601
18. Mossman T (1983) Rapid colometric assay for cellular growth and survival: application to proliferation and cytotoxic assays. J Immunol Methods 65: 55–59
19. Onoda J, Nelson K, Taylor J, Honn K (1989) In vivo characterization of combination antitumor chemotherapy with calcium channel blockers and cis-diamminedichloroplatinum (II). Cancer Res 49: 2844
20. Parker R, Eastman A, Bostc-Bruton F, Reed E (1991) Acquired cisplatin resistance in human ovarian cancer cells is associated with enhanced repair of cisplatin-DNA lesions and reduced drug accumulation. J Clin Invest 87: 772
21. Phillips T, Levin V, Ahn D, Gubn P, Davis R, Wilson C, Prados M, Wara W, Flam M (1991) Evaluation of bromo-de-oxyuridine in glioblastoma multiforme: a Northern California Cancer Center Phase 11 Study. Int J Radiat Oncol Biol Phys 21: 709
22. Russo A, DeGraff W, Kinsella TJ, Gamson J (1986) Potentiation of chemotherapy cytotoxicity following iododeoxy-uridine incorporation in Chinese hamster cells. lnt J Radiat Oncol Biol Physics 12: 1371
23. Saikawa Y, Kubota T, Kuo T, Tanino H, Kase S, Furukawa T, Watanabe M, lshibiki K, Kitajima M, Hoffman R (1994) Combined effect of 5-fluorouracil and carboplatin against human gastric cancer cell lines in vitro and in vivo. Anticancer Res 14: 461
24. Scanlon D, Newman E, Lu Y, Priest D (1984) Biochemical basis for cisplatin and 5-fluorouracil synergism in human ovarian carcinoma cells. Proc Natl Acad Sci USA 83: 8923
25. Shimoyama Y, Kubota T, Watanabe M, lshibiki K, Abe O (1989) Predictability of in vivo chemosensitivity by in vitro MTT assay with reference to the clonogenic assay. J Surg Oncol 41: 12
26. Tochner Z, Kinsella T, Rowland J, Gatstein (1989) Treatment of unresectable sarcomas of adults with hyper-fractionated irradiation and iododeoxyuridine. In: McNally, N.J., ed. Scientific basis of modern radiotherapy, BIR Report No. 19, London, UK: British Institute of Radiology, p 107
27. Vokes E, Ratain M, Mick R, McEvilly J, Haraf D, Kozlof F, Hamasaki V, Weichselbaum R, Panie W, Weing B, Berenz F (1993) Cisplatin, fluorouracil and leucovorin augmented by interferon alpha-2b in head and neck cancer: a clinical and pharmacologic analysis. J Clin Oncol 11: 360