

Proliferation Indices as Molecular Pharmacodynamic Endpoints in Evaluation of Anticancer Drug Effect in Human Solid Tumors

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Received May 14, 1998; accepted July 13, 1998

Purpose. The present study compared proliferative indices, i.e. incorporation of DNA precursor (i.e. thymidine or TdR, and bromodeoxyuridine or BrdU) and expression of proliferating cell nuclear antigen (PCNA), as molecular pharmacodynamic endpoints in evaluation of anticancer drug effect in human solid tumors.

Methods. Tumor specimens obtained from patients were grown as histocultures. After treatment with doxorubicin, mitomycin C, and/or paclitaxel, cells labeled by [³H]TdR were identified using autoradiography, and cells labeled by BrdU and PCNA were identified using immunohistochemical techniques. Drug effect was measured as reduction of DNA precursor-labeled cells or PCNA-expressing cells.

Results. The results indicate that (a) the two DNA precursors, TdR and BrdU, labeled the same cells and resulted in identical pharmacodynamics, (b) the pharmacodynamics established using inhibition of DNA precursor incorporation were qualitatively and quantitatively different from the pharmacodynamics established using inhibition of PCNA expression, (c) the inhibition of PCNA expression was erratic in some tumors, and (d) the differences in pharmacodynamics established using the two end points are drug-specific, with greater differences for paclitaxel than for mitomycin C.

Conclusions. The erratic results measured by the PCNA labeling method suggest that this method may be less reliable than the conventional DNA precursor labeling method. The finding of identical pharmacodynamics of doxorubicin and paclitaxel established using BrdU and [³H]TdR indicates that the two precursors are interchangeable. Because the methodology for detecting BrdU incorporation requires less time and does not require the use of radioactivity, we conclude that inhibition of BrdU incorporation represents a useful endpoint for evaluating the antiproliferative activity of anticancer drugs in human solid tumors.

KEY WORDS: proliferative indices; molecular pharmacodynamic endpoints; anticancer drugs; human solid tumors; thymidine; bromodeoxyuridine.

INTRODUCTION

Incorporation of DNA precursors is considered the gold-standard of proliferation index and has been used to determine cell proliferation in studying chemical carcinogenesis (1) and cell kinetics (2), to determine the fraction of S phase cells (3), and to predict tumor progression (4). Several reports have shown

that the two DNA precursors, i.e. [³H]thymidine ([³H]TdR) and bromodeoxyuridine (BrdU), are interchangeable in labeling proliferating cells (1,5–7). Inhibition of DNA precursor incorporation is a commonly used endpoint for evaluating the effect of anticancer agents. Our laboratory has used this method to measure the antiproliferative effect of anticancer drugs in human solid tumors (e.g. 8–10). The clinical relevance of this method has been demonstrated in retrospective and semi-prospective preclinical and clinical studies showing that the drug response in patient tumors correlates with the sensitivity and resistance of cancer patients to chemotherapy as well as patient survival (11–13). This method has several limitations. First, the long period required for the cumulative incorporation of DNA precursors (i.e. 4 day) is time-consuming and limits the flexibility of experimental design. For example, the drug effect immediately after treatment cannot be determined. Second, the method requires exposing live cells to DNA precursor and, hence, does not apply to archival tissues. Third, the use of radioactive DNA precursor such as [³H]TdR poses environmental hazards and is associated with high costs of radioactivity disposal. Fourth, the autoradiographic procedures used to measure [³H]TdR incorporation require 5 to 7 day for processing.

Endogenous proliferation markers, i.e. cellular molecules that are present only in cycling cells, offer several theoretical advantages over DNA precursors. First, measurement of endogenous markers can be performed on archival tissues. Second, there is no need to expose live cells to DNA precursors and, hence, the several problems associated with DNA precursor labeling are avoided. Endogenous proliferation markers such as mitotic figures, nucleolar organizing regions, proliferating cell nuclear antigen (PCNA) and Ki-67 have been used to identify proliferating cells (14–17). PCNA was originally referred to as cyclin because of its cyclic expression pattern; PCNA protein levels rise in late G₁ phase, reach maximal levels in mid-S phase and decline in M phase (18). PCNA represents an attractive choice because of its stability in paraffin-embedded specimens and the ease of detection with immunohistochemistry. Similar to DNA precursor incorporation, PCNA expression has shown value as a prognostic indicator of tumor recurrence or progression (5,19,20). PCNA has a half-life of about 20 hr (21). PCNA labeling has been correlated with DNA precursor labeling in various tissues (22–24). This correlation suggests PCNA labeling as a potential new endpoint for studying the antiproliferative effect of drugs. However, because PCNA expression and DNA precursor (i.e. BrdU) incorporation occur in different subpopulations in different cell lines (25), it is not known whether the two end points yield identical pharmacodynamics.

The present study compared DNA precursor incorporation and PCNA expression as pharmacodynamic endpoints, and compared two different DNA precursors, i.e. TdR and BrdU. We have previously shown a linear correlation between PCNA labeling and TdR labeling in human bladder tumors (26). This study used 3-dimensional histocultures of human bladder and head and neck tumors, and three anticancer drugs with different action mechanisms and qualitatively different pharmacodynamics, i.e. doxorubicin which is a topoisomerase II inhibitor, mitomycin C (MMC) which is an alkylating agent, and paclitaxel which is a tubulin-binding agent.

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ABBREVIATIONS: BrdU, bromodeoxyuridine; IC, drug concentration required to inhibit DNA precursor incorporation or PCNA expression; LI, labeling index; MEM, Minimum Essential Medium; MMC, mitomycin C; PBS, phosphate-buffered saline; PCNA, proliferating cell nuclear antigen; TdR, thymidine.

MATERIALS AND METHODS

Chemicals and Supplies

MMC and paclitaxel were gifts from Bristol Myers Squibb (Princeton, NJ), and doxorubicin from Adria Laboratories (Columbus, OH). Cefotaxime sodium was purchased from Hoechst-Roussel (Somerville, NJ), gentamycin from Solo Pak Laboratories (Franklin Park, IL), Minimal Essential Medium (MEM), nonessential amino acids, fetal bovine serum and glutamine from GIBCO Laboratories (Grand Island, NY), sterile pigskin collagen (Spongostan standard) from Health Designs Industries (Rochester, NY), NTB-2 nuclear track emulsion and D-19 high contact developer from Eastman Kodak Co (Rochester, NY), BrdU from Sigma Co. (St. Louis, MO), [³H]TdR from ICN Biomedicals (Irvine, CA), antibody against PCNA and the LSAB (linked streptavidin-biotin immunoperoxidase kit) from Dako (Carpinteria, CA), and the 3,3'-diaminobenzidine kit and antibody against BrdU from BioGenex (San Ramon, CA). All chemicals and reagents were used as received.

Tumor Specimens

Tumor specimens were provided by the Tumor Procurement Service at The Ohio State University Comprehensive Cancer Center. Primary human bladder tumors were obtained via transurethral resection or cystectomy. Head and neck specimens were taken from the primary sites or the cervical lymph nodes. Within 10 to 30 min after surgery, tumor specimens were placed in MEM or Hank's balanced salt solution and maintained at 4°C until use.

Pharmacodynamic Studies

Histoculture of tumors was performed as previously described (8–10). Briefly, specimens were dissected into 1 mm³ pieces and five randomly chosen tumor fragments were placed on a 1 cm² piece of pre-hydrated collagen gel. Tissue specimens were cultured in 6-well plates in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. The culture medium consisted of MEM fortified with 9% heat-inactivated fetal bovine serum, 0.1 mM nonessential amino acids, 100 µg/ml gentamycin, 95 µg/ml cefotaxime, and 2 mM glutamine, and was adjusted to a pH of 7.4.

After culture for at least one day, the culture media was exchanged for drug-containing media. Bladder tumors were treated with paclitaxel (0.01 nM to 10 µM), doxorubicin (10 nM to 100 µM), or MMC (300 nM to 300 µM) for two hr, which is the period of intravesical treatment in patients. Head and neck tumors were treated with only paclitaxel (0.01 nM to 10 µM) for 24 hr, which is the infusion duration in patients. After drug treatment, tumor pieces were rinsed three times with 5 ml of drug-free medium and then cultured with [³H]TdR (60 Ci/mmol, 1 µCi/ml) for 4 day or BrdU (40 µM) for 2 day. To determine if [³H]TdR and BrdU labeled the same cells, some tumors were exposed simultaneously to the two precursors for 2 day. Afterwards, specimens were fixed for a minimum of 24 hr in 10% neutral buffered formalin. Five µm sections of the paraffin-embedded tissues were mounted on slides and the proliferating cells were identified by autoradiography for [³H]TdR labeling or immunohistochemistry for BrdU and PCNA labeling (see below). Control tissues were treated similarly, but were not treated with drugs.

Autoradiography

Slides for autoradiography were dried overnight at 60°C, to allow tissues to adhere to the glass. The tissue sections were deparaffinized, hydrated, stained with hematoxylin and exposed to autoradiographic emulsion for 5 day at 4°C. The emulsion was developed with high contrast developer and fixed with sodium thiosulfate (20% w/v). Slides were counterstained with eosin, dehydrated and coverslipped.

Immunohistochemical Detection of PCNA and BrdU

Tissue sections were mounted on poly L-lysine-coated slides and dried overnight at room temperature. After the tissue sections were deparaffinized and hydrated, the PCNA antigen was unmasked by boiling the slides in 800 ml of antigen retrieval solution for 10 min in a microwave, and the BrdU antigen by boiling the slides in 700 ml citrate buffer (10 mM citric acid, pH 6.0) for 5 min. Following antigen retrieval, the slides were cooled for 15 min and rinsed in phosphate buffered saline (PBS). Immunohistochemical detection of PCNA and BrdU was performed using the LSAB kit. Briefly, non-immune goat serum was applied to tumors for 10–20 min to block nonspecific binding sites. After excess goat serum was removed, the anti-BrdU antibody diluted in bovine serum albumin (5 mg/ml) or the anti-PCNA antibody diluted in 1% dry milk, was applied to tumors at room temperature for 30 and 45 min, respectively. After rinsing in PBS, biotinylated linker antibody and streptavidin-peroxidase were applied sequentially for 20 min each, followed by rinses with PBS. Peroxidase activity was detected with 3,3'-diaminobenzidine after incubation for 3 min for BrdU or 8 min for PCNA. Slides were rinsed in water and counterstained with hematoxylin.

For tumors that were labeled with both [³H]TdR and BrdU, the slides were first processed for BrdU labeling using immunohistochemical detection, followed by processing for [³H]TdR labeling using autoradiography.

Evaluation of Labeling

Tumor slides were scanned microscopically at 100× magnification to select the field with the highest percentage of labeled cells. The total number of cells and the cells labeled for PCNA or DNA precursors were counted under 400× magnification. Nuclei covered by at least 10 silver autoradiographic grains were considered [³H]TdR-labeled; brown nuclei were considered positive for PCNA or BrdU. Labeling index (LI), defined as the percentage of labeled cells, was determined for each tumor piece. Approximately 100 to 200 cells were evaluated per tumor piece.

Pharmacodynamic Data Analysis

Drug effect was measured by the inhibition of PCNA and DNA precursor LI. The doxorubicin or MMC concentration-effect relationship was analyzed by computer-fitting Equation 1 to the experimental data, while Equation 2 was used for the paclitaxel data (8–10).

$$E = E_0 \left(1 - \frac{C^n}{K^n + C^n} \right) \quad (1)$$

$$E = (E_0 - Re) * \left(1 - \frac{C^n}{K^n + C^n} \right) + Re \quad (2)$$

where E is the LI of drug-treated tissues expressed as a percentage of the LI of control tissues, E_0 is the baseline LI in the absence of drug, C is the drug concentration, K is the concentration at one-half E_0 , n is a curve shape parameter and Re is the residual fraction of labeled cells at the highest drug concentrations. Inclusion of the Re term is necessary to describe the less-than-complete effect of paclitaxel on inhibition of DNA synthesis.

Statistical Analysis

Differences in the pharmacodynamic parameters obtained using the two DNA precursors were analyzed using two-tailed paired t-test.

RESULTS

PCNA and DNA Precursor Labeling in Human Tumors

A total of 29 tumors, including 23 bladder tumors and 6 head and neck tumors, were studied. Eight tumors, including 5 bladder and 3 head and neck tumors, were used to compare the pharmacodynamics determined using [^3H]TdR or BrdU as the DNA precursors. The bladder tumors in this group were treated with doxorubicin and head and neck tumors were treated with paclitaxel. Twenty-one additional tumors, including 18 bladder and 3 head and neck tumors, were used to compare the inhibition of DNA precursor incorporation and inhibition of PCNA expression as the pharmacodynamic endpoint. For this group, 10 of the 18 bladder tumors were treated with MMC only (Tumor number 9 through 18), 3 were treated with paclitaxel and MMC in separate treatments (Tumor number 19 through 21), and 5 were treated with paclitaxel only (Tumor number 22 through 26). The 3 head and neck tumors were treated only with paclitaxel (Tumor number 27 through 29).

Figure 1 shows the labeling of PCNA-expressing cells and the cells that incorporated DNA precursors. DNA precursor labeling was typically all-or-none, resulting in minimal variation in the intensity of color or autoradiographic signals for labeled cells. In contrast, the intensity of PCNA staining varied by several fold within a tumor and between different tumors. In the 8 tumors that were doubly labeled with [^3H]TdR and BrdU, >98% of the labeled cells were labeled by both precursors, indicating that the two DNA precursors labeled the same cells (Fig. 1D). The PCNA LI and DNA precursor LI in untreated controls were comparable for both bladder tumors (i.e. mean \pm SD of $49.8 \pm 24.5\%$ vs $51.9 \pm 19.5\%$) and head and neck tumors (i.e. $53.0 \pm 26.7\%$ vs $44.1 \pm 19.0\%$).

Pharmacodynamics Measured by Inhibition of DNA Precursor Incorporation and PCNA Expression

Figure 2 and Table 1 show the pharmacodynamics of doxorubicin and paclitaxel, measured by inhibition of either [^3H]TdR or BrdU incorporation. The comparison indicates insignificant differences in the results obtained using either DNA precursors, consistent with the above finding that the two precursors labeled the same cells. In contrast, there are qualitative and quantitative differences in the pharmacodynamic data obtained using inhibition of either DNA precursor incorporation or PCNA expression. For MMC, the pharmacodynamic relationship showed a classical sigmoidal-shaped curve in 12 of 13 tumors when

inhibition of DNA precursor incorporation was used as the endpoint, and in 9 of 13 tumors when inhibition of PCNA expression was used as the endpoint. Two examples are shown in Fig. 3. One tumor shows sigmoidal curves for both endpoints. The other tumor shows a sigmoidal curve for drug-induced inhibition of DNA precursor incorporation, but no relationship between drug concentration and inhibition of PCNA expression. Table 2 summarizes the MMC pharmacodynamics in individual tumors. The two endpoints yielded similar maximal inhibition. However, there are substantial differences in the IC_{50} values determined using the two endpoints, with some tumors showing higher IC_{50} values obtained using inhibition of PCNA expression, whereas other tumors showed the opposite.

While the differences in the MMC pharmacodynamics as measured by the two endpoints are mainly quantitative (i.e. different IC_{50} values), the paclitaxel pharmacodynamics measured by inhibition of DNA precursor incorporation are qualitatively and quantitatively different from the pharmacodynamics measured by inhibition of PCNA expression. The PCNA measurement did not result in sigmoidal pharmacodynamic relationship in all of 11 tumors, whereas the DNA precursor measurement resulted in a sigmoidal relationship in 4 of 11 tumors. Table 3 summarizes the pharmacodynamics of paclitaxel in individual tumors. The two endpoints yielded different maximal inhibition, with a higher value for the DNA precursor measurement compared to the PCNA measurement. We did not compare the IC_{50} values obtained by the two methods, because we could not obtain IC_{50} values from the PCNA measurement due to its erratic concentration-effect relationship.

Collectively, the results indicate that the pharmacodynamics established using inhibition of PCNA expression are more variable and less likely to follow the commonly observed sigmoidal-shaped concentration-response relationship, compared to inhibition of DNA precursor incorporation.

DISCUSSION

The present study compared the pharmacodynamics of doxorubicin, MMC, and paclitaxel using different proliferative indices. The results indicate (a) identical pharmacodynamics in the inhibition of the incorporation of two DNA precursors (i.e. TdR and BrdU), (b) qualitative and quantitative differences in the pharmacodynamics established using inhibition of DNA precursor incorporation and inhibition of PCNA expression, (c) the inhibition of PCNA expression was erratic in some tumors, and (d) that the differences in pharmacodynamics established using the two end points are drug-specific, with greater differences for paclitaxel than for MMC. The last finding suggests that the different results obtained with the two endpoints may be due to differences in the mechanisms of drug action.

There are several possible reasons that inhibition of PCNA expression frequently did not provide a concentration-dependent drug effect. First, PCNA expression is deregulated and shows significant variability in tumors (27). Second, PCNA is involved in DNA excision repair (28), which may lead to variation in PCNA expression after treatment with MMC, a DNA damaging agent. Third, the two methods may target different cells. For example, PCNA expression and BrdU incorporation have been shown to occur in different subpopulations in different cell lines (25). The DNA precursor incorporation method targets cells that are in the S phase undergoing DNA

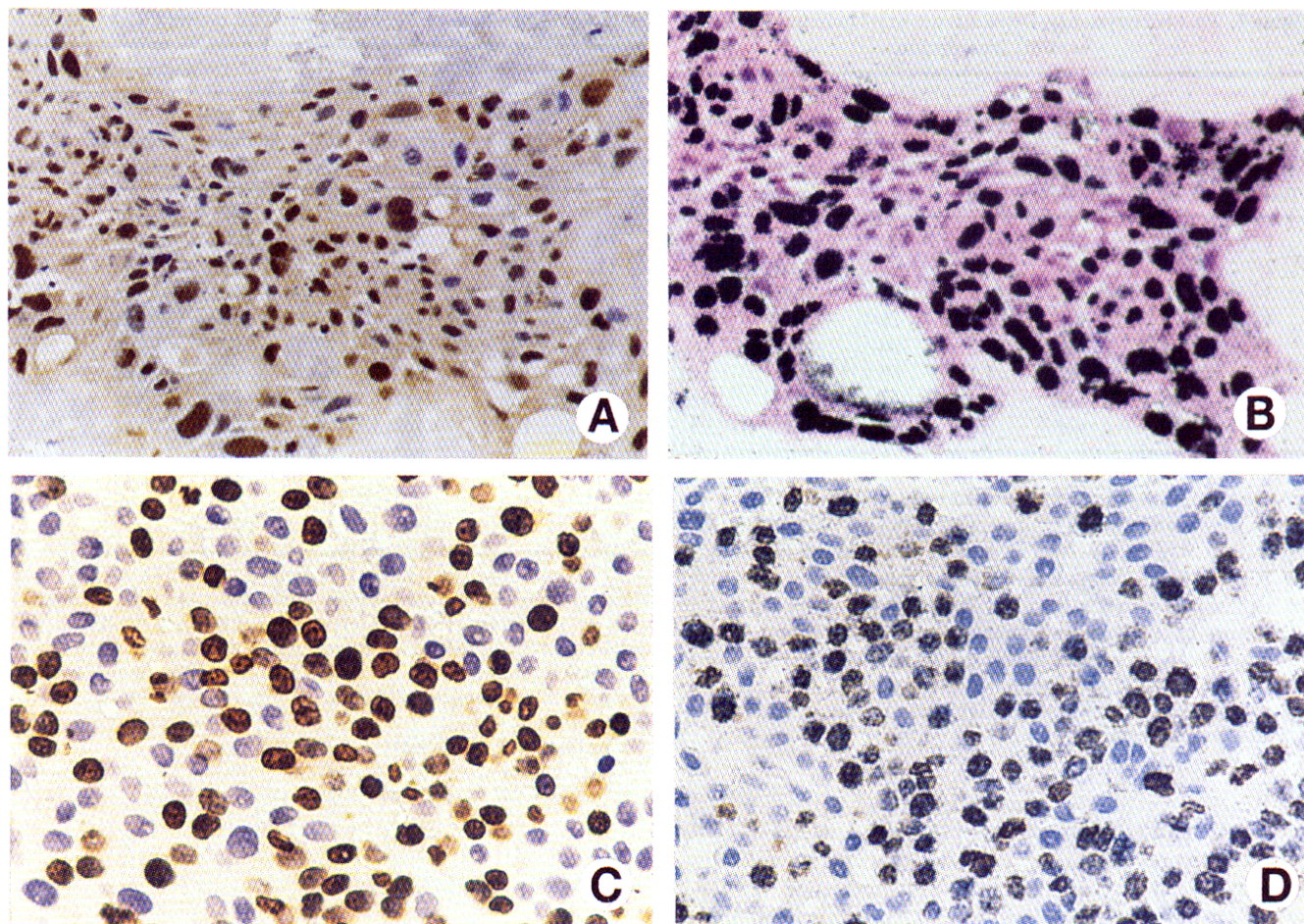


Fig. 1. PCNA and DNA precursor labeling. (A) A head and neck tumor labeled for PCNA (brown stain), 200 \times magnification. (B) A head and neck tumor labeled for [^3H]TdR (black grains), 200 \times magnification. (C) A bladder tumor labeled for BrdU (brown stain), 400 \times magnification. (D) A bladder tumor labeled for both [^3H]TdR and BrdU. 400 \times magnification. The tissues were counterstained with hematoxylin (A, C, and D) or hematoxylin and eosin (B).

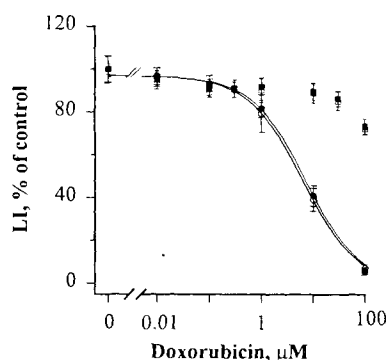


Fig. 2. Pharmacodynamics of Doxorubicin and paclitaxel measured by inhibition of [^3H]TdR or BrdU incorporation. Human bladder and head and neck histocultures were treated with doxorubicin for 2 and paclitaxel for 24 hr, respectively. Drug effect was determined by the inhibition of incorporation of [^3H]TdR (\circ , \square) or BrdU (\bullet , \blacksquare). Examples of bladder tumors that showed (\circ , \bullet) and did not show (\square , \blacksquare) a sigmoidal concentration-effect relationship after doxorubicin treatment (Tumor #5 and #3, respectively). Mean \pm SEM. Lines are computer-fitted concentration-response curves according to Equation 1. Note that the data on Tumor #3 could not be fitted with Equation 1.

synthesis. On the other hand, there are two populations of PCNA, in chromatin and in nucleoplasm. The chromatin PCNA is actively involved in DNA replication and repair, whereas the nucleoplasmic PCNA functions as a reserve for the chromatin PCNA. The PCNA expression method, because we used formalin-fixed tissues (which preserves both the PCNA in the nucleoplasm and bound to the chromatin, ref. 21), identifies all cells in the cell cycle, including G₁, S, M and G₂ phase cells. It is conceivable that specific labeling of the chromatin PCNA which is expressed in cells undergoing DNA synthesis may yield results that are more similar to the DNA precursor incorporation data. The preservation of chromatin PCNA requires the use of alcohol instead of formalin as the fixative agent. The less popular alcohol fixation method poses the disadvantage of distortion of tissue architecture, which may interfere with micrographic evaluation.

In conclusion, our results show that inhibition of nucleoplasm PCNA expression is not suitable for evaluating the effect of anticancer drugs in human solid tumors, while the inhibition of DNA precursor provides well-defined pharmacodynamics in most tumors. The results further show identical pharmacodynamics of doxorubicin and paclitaxel established using the two DNA precursors, BrdU and [^3H]TdR. Because BrdU incorpora-

Table 1. Pharmacodynamics Determined Using Inhibition of [³H]TdR or BrdU Incorporation

Tumor	[³ H]TdR LI			BrdU LI		
	Maximal inhibition (%)	Sigmoidal response	IC ₅₀ (μM)	Maximal inhibition (%)	Sigmoidal response	IC ₅₀ (μM)
1	100	yes	1.4	100	yes	1.7
2	95	yes	5.4	93	yes	4.7
3	18	no ^a	NA	16	no ^a	NA
4	13	no ^a	NA	10	no ^a	NA
5	97	yes	7.5	96	yes	6.9
6	70	yes	2.1	65	yes	2.0
7	46	no ^a	NA	43	no ^a	NA
8	29	no ^a	NA	31	no ^a	NA

Note: Human bladder tumors were treated with doxorubicin for 2 hr and head and neck tumors were treated with paclitaxel for 24 hr. The tumors were then labeled simultaneously with [³H]TdR and BrdU for 96 hr. The same microscopic fields were analyzed for inhibition of [³H]TdR and BrdU incorporation. Maximal inhibition was achieved at the highest concentration used (i.e. 100 μM for doxorubicin and 10 μM for paclitaxel). Tumors 1 through 5 are bladder tumors. Tumors 6 through 8 are head and neck tumors. In tumors which showed less than 50% inhibition, the IC₅₀ could not be determined. NA, not applicable. ^a Showed concentration-dependent inhibition but not a complete sigmoidal-shaped relationship.

tion requires only 2 day exposure to BrdU whereas [³H]TdR incorporation requires 4 day exposure plus lengthy autoradiographic procedures, and because BrdU detection does not require the use of radioisotope, we conclude that inhibition of BrdU incorporation represents a useful endpoint for evaluating the antiproliferative activity of anticancer drugs in human solid tumors.

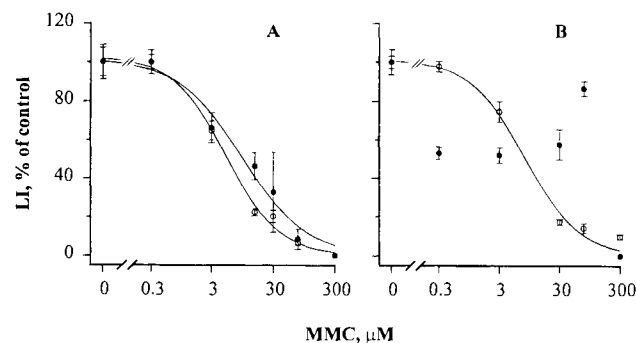


Fig. 3. MMC pharmacodynamics measured by inhibition of PCNA expression and DNA precursor incorporation. Human bladder tumor histocultures were treated with MMC for 2 hr. Drug effect was determined by the inhibition of incorporation of DNA precursors (○), or labeling of PCNA (●). (A) Example of a tumor which showed sigmoidal concentration-effect relationship when drug effect was measured as inhibition of PCNA expression or DNA precursor incorporation (Tumor #13). (B) Example of a tumor which showed sigmoidal relationship when inhibition of DNA precursor was used as the endpoint, but an erratic concentration-dependent effect when inhibition of PCNA expression was used as the endpoint (Tumor #18). Mean ± SEM. Lines are computer-fitted concentration-response curves according to Equation 1. Note that for Panel B, the PCNA data could not be fitted with Equation 1.

Table 2. Inhibition of PCNA and [³H]TdR or BrdU LI by MMC

Tumor	PCNA LI			DNA precursorLI		
	Maximal inhibition (%)	Sigmoidal response	IC ₅₀ (μM)	Maximal inhibition (%)	Sigmoidal response	IC ₅₀ (μM)
9	100	yes	37.3	100	yes	14.2
10	100	no ^b	NA	100	yes	2.1
11	100	yes	3.7	99	yes	8.7
12	100	yes	100	90	yes	33.2
13	100	yes	9.5	100	yes	5.2
14	95	yes	97	97	yes	20.7
15	100	no ^b	NA	92	yes	1.5
16	100	yes	17.2	99	yes	8.5
17	100 ^a	yes	3.9	100 ^a	yes	100
18	99	no ^b	NA	97	yes	7.5
19	99	yes	22	61	yes	4.0
20	none	no ^b	NA	94	no ^b	NA
21	89	yes	22.7	100	yes	26.6

Note: Human bladders tumors were treated with MMC for 2 hr and the drug effect was analyzed as inhibition of DNA precursor incorporation or inhibition of PCNA expression. Maximal inhibition was achieved at the highest concentration used (i.e. 300 μM) unless otherwise noted. In tumors which showed less than 50% inhibition or no concentration-dependent inhibition, the IC₅₀ could not be determined. NA, not applicable.

^a Maximal inhibition achieved at 150 μM.

^b Showed erratic concentration-effect relationship, i.e. not concentration-dependent or sigmoidal-shaped.

ACKNOWLEDGMENTS

This study was supported in part by R37CA49816 and R01CA63363 from the National Cancer Institute, NIH, DHHS. The Tumor Procurement Service of the Ohio State University

Table 3. Inhibition of PCNA and [³H]TdR or BrdU LI by Paclitaxel

Tumor	PCNA LI			DNA precursor LI		
	Maximal inhibition (%)	Sigmoidal response	IC ₅₀ (nM)	Maximal inhibition (%)	Sigmoidal response	IC ₅₀ (nM)
19	15	no ^a	NA	60	yes	1,000
20	0	no ^a	NA	38	no ^a	NA
21	0	no ^a	NA	73	yes	264
22	0	no ^a	NA	0	no ^a	NA
23	0	no ^a	NA	0	no ^a	NA
24	0	no ^a	NA	0	no ^a	NA
25	35	no ^a	NA	0	no ^a	NA
26	33	no ^a	NA	47	no ^a	NA
27	25	no ^a	NA	81	yes	38.5
28	85	no ^a	NA	52	no ^a	NA
29	0	no ^a	NA	60	yes	1,669

Note: Human bladder and head and neck tumors were treated with paclitaxel for 2 or 24 hr, respectively. Maximal inhibition was the highest inhibition of PCNA expression or DNA precursor incorporation at any concentration. Tumors 19 through 26 are bladder tumors. Tumors 27 through 29 are head and neck tumors. In tumors which showed less than 50% inhibition or no concentration-dependent inhibition, the IC₅₀ could not be determined. NA, not applicable.

^a Showed erratic concentration-effect relationship, i.e. not concentration-dependent or sigmoidal-shaped.

Comprehensive Cancer Center was supported by P30CA16058 from the National Cancer Institute. We gratefully acknowledge Chong Lim, and Drs. Thomas Schmittgen and John Kalns for providing the archival tumor samples.

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