Time- and Concentration-Dependent Penetration of Doxorubicin in Prostate Tumors

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ABSTRACT The penetration of paclitaxel into multilayered solid tumors is time- and concentration-dependent, a result of the drug-induced apoptosis and changes in tissue composition. This study evaluates whether this tissue penetration property applies to other highly protein-bound drugs capable of inducing apoptosis. The penetration of doxorubicin was studied in histocultures of prostate xenograft tumors and tumor specimens obtained from patients who underwent radical prostatectomy. The kinetics of drug uptake and efflux in whole tumor histocultures were studied by analyzing the average tumor drug concentration using high-pressure liquid chromatography. Spatial drug distribution in tumors and the drug concentration gradient across the tumors were studied using fluorescence microscopy. The results indicate that drug penetration was limited to the periphery for 12 hours in patient tumors and to 24 hours in the more densely packed xenograft tumors. Subsequently, the rate of drug penetration to the deeper tumor tissue increased abruptly in tumors treated with higher drug concentrations capable of inducing apoptosis (i.e., >5 μm), but not in tumors treated with lower concentrations. These findings indicate a time- and concentration-dependent penetration of doxorubicin in solid tumors, similar to that of paclitaxel. We conclude that doxorubicin penetration in solid tumors is time- and concentration-dependent and is enhanced by drug-induced cell death.

Key words: Doxorubicin, Delivery, Apoptosis, Solid Tumor

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INTRODUCTION

Drug delivery to a solid tumor is governed by several factors that differ for systemic and regional treatments. Following a systemic intravenous injection, drug delivery to the tumor core involves 3 processes (ie, distribution through vascular space, transport across microvessel walls, and diffusion through interstitial space in tumor tissue) (1). When the drug is directly injected into a tumor, such as by intratumoral injection or by direct instillation into peritumoral space as in intravesical therapy of superficial bladder cancer and in intraperitoneal dialysis of ovarian cancer, drug delivery to tumor cells is primarily by diffusion through interstitial space (2-6).

The inability of a drug to penetrate a solid tumor is considered resistance of solid tumors to anticancer drugs (7-11). For example, penetration of doxorubicin in 3-dimensional tumor cell spheroids after 1 to 2 hours is limited to the periphery (7-9,12). Similarly, a steep concentration gradient in breast tumors has been observed in patients (13). Hence, a better understanding of the determinants of drug penetration into solid tumors is needed.

We recently studied the penetration of paclitaxel, a highly protein-bound drug, in solid tumors. The study was performed under in vitro conditions where paclitaxel was placed in the culture medium surrounding histocultures of tumor fragments (~1 mm^3). The results show that a high tumor cell density is a barrier to paclitaxel penetration in tumor tissue; paclitaxel penetration is restricted to the tumor periphery until the cell density is reduced as a result of paclitaxel-induced apoptosis, at which time paclitaxel distributes evenly throughout the tumor (14). This study examined whether the time-dependent and apoptosis-enhanced drug delivery applies to other drugs. We examined several aspects of doxorubicin penetration into solid tumors (ie,

kinetics of drug penetration and effects of tumor cell density and tissue composition on drug penetration). Doxorubicin, similar to paclitaxel, is a highly protein-bound drug. The study was performed using histocultures of prostate tumors obtained from patients and human xenograft tumors maintained in immunodeficient mice. Histocultures are fragments of tumors obtained from a human or animal host, cut to approximately 1 mm^3 and cultured on a collagen matrix. Histocultures maintain a 3-dimensional structure and, therefore, cell-cell interaction and clonal heterogeneity. This is similar to spheroids, which are aggregates of cultured tumor cells, sometimes cocultured with fibroblasts (15,16). We preferred using histocultures for the current study because this allows us to study drug penetration in patient tumor material and because the results obtained using patient tumors are more likely to be clinically relevant. The presence of stromal cells and matrix material is considered important for prostate tumor growth (17) and, as shown in this study, plays a role in the penetration and accumulation of doxorubicin in prostate tumor. In addition, the clinical relevance of the histoculture system has been demonstrated in retrospective and semiprospective preclinical and clinical studies; drug response in human tumor histocultures correlates with chemosensitivity and survival of cancer patients to several chemotherapeutic drugs (18-20).

MATERIALS AND METHODS

Chemicals and Supplies

Doxorubicin and epirubicin were gifts from Pharmacia & Upjohn (Milan, Italy; Albuquerque, NM) or purchased from Sigma Co (St Louis, MO). Male athymic BALB/C Nu/Nu mice were purchased from the National Cancer Institute (Frederick, MD); cefotaxime sodium from Hoechst-Roussel Inc (Somerville, NJ); gentamicin from Solo Pak Laboratories (Franklin Park, IL); fetal bovine serum (FBS), nonessential amino acids, L-glutamine, minimum essential medium (MEM), Dulbecco's Modified Eagle Medium (DMEM), and RPMI 1640 medium from GIBCO Laboratories (Grand Island, NY); sterile pigskin collagen gel (Spongostan standard) from Health Designs Industries (Rochester, NY); cryotome imbedding polymer from Miles Inc (Ellchart, IN); solid phase extraction tubes (Supelclean LC-18) from Supelco (Bellefonte, PA); a rotor-stator type of tissue homogenizer (Tissumizer) from Tekmar (Cincinnati, OH); and Pecosphere reversed-phase C_{18} columns (3 μm particle size, 83 mm X 4.6 mm) from Perkin-Elmer (Norwalk, CT).

Tumor procurement

Surgical specimens of human prostate tumors were obtained through the Tumor Procurement Service at The Ohio State University Comprehensive Cancer Center from patients who underwent radical prostatectomy. Tumor specimens were placed in MEM within 10 to 30 minutes after surgical excision, stored on ice, and prepared for culturing within 1 hour after excision.

Human prostate tumor xenografts maintained in nude mice.

The two human prostate xenograft tumors (ie, the androgen-dependent CWR22 and the androgen-independent PC3 tumors) were established and maintained as described previously (21-23). Briefly, minced tumor tissue was mixed with an equal volume of Matrigel, and 0.3 mL of the mixture was implanted into both flanks of a mouse. Tumors were harvested when they reached a size of 1 g at about 7 weeks for CWR22; 4 weeks for PC3. For the CWR22 tumor, animals were implanted subcutaneously with a testosterone pellet (12.5 mg/tablet, Innovative Research of America, Toledo, OH) 3 days before tumor implantation.

Histocultures

Patient prostate or tumor xenograft specimens were processed as previously described (14). Briefly, specimens were washed 3 times and dissected into fragments measuring about 1 mm^3. The culture medium consisted of MEM/DMEM (1:1) for patient tumors, or RPMI 1640 for PC3 xenograft tumor, supplemented with 9% heat-inactivated FBS, 2 mM l-glutamine, 0.1 mM nonessential amino acids (only for MEM/DMEM), 90 μg/ml gentamicin and 90 μg/mL cefotaxime sodium. For the CWR22 xenograft tumor, the culture medium consisted of a 1:1 mixture of MEM and DMEM, 10% fetal bovine serum, 0.1 mM nonessential amino acids, 2 mM l-glutamine, 1 mM sodium pyruvate, MEM vitamin solution, and 40 μg/mL gentamicin.

Drug uptake and efflux in histocultures

Histocultures were placed on a 1-cm^2 piece of presoaked collagen gel (5 histocultures per gel) and incubated with 4 mL culture medium in 6-well plates. The culture medium was refreshed every other day. After 3 to 4 days, the histocultures were treated with 0.02 to 20 μM doxorubicin for up to 96 hours. We have shown that these concentrations were sufficient to inhibit proliferation and induce cell death in patient prostate tumors (23). For the efflux study, tumor histocultures were incubated with doxorubicin for 96 hours. The drug-containing medium was then exchanged with drug-free medium, and histocultures and aliquots of medium were collected at predetermined times. The histocultures were blot-dried on filter paper and weighed.

For each tumor, 3 to 5 histocultures were used for each concentration and each time point. The study design of experiments using patient tumors was dictated by the size of the specimens. On some occasions, specimens from an individual patient were only sufficient to study drug uptake and efflux at 1 or more, but not all, drug concentrations. Ten patient tumors were used. For the xenograft tumors, specimens from individual animals were sufficiently large that each tumor was used for studying uptake and efflux at all drug concentrations.

HPLC analysis of doxorubicin concentration

The concentration of doxorubicin in culture medium was analyzed by high-pressure liquid chromatography (HPLC); the concentration of doxorubicin in tumors was analyzed by HPLC and quantitative fluorescence microscopy. Drug concentration in tissue was calculated as (drug amount) divided by (tissue weight) and was expressed in molar terms.

For HPLC analysis, we used previously published methods (24,25) with minor modifications, as follows. Epirubicin was used as the internal standard and was added before sample extraction. For tumor histocultures, samples (average weight of ~5 mg) were homogenized for 1 minute with 2 mL acidified methanol (5% of 50 mM potassium phosphate buffer [pH 3.0] in methanol). Homogenates adhering to the homogenizer were recovered by rinsing with 3 mL of methanol. The methanolic extract was reduced to a volume of less than 2 mL by evaporation, followed by mixing with 3 mL of 10 mM potassium phosphate, pH 8.0, and 2 mL methanol. The final mixture was loaded on a C18 solid phase extraction column, which was preconditioned with 3 mL 100% methanol, followed by 3 mL of a 1:3 mixture of methanol:20 mM potassium phosphate, pH 8.0. After washes with 1 mL of water followed by 2 mL of 50% methanol in water, the analytes were eluted with 6 mL of a 95:5 mixture of methanol:50 mM potassium phosphate, pH 3.0. The extract was then evaporated to dryness. The residue was reconstituted with the mobile phase and analyzed by HPLC.

For the analysis of doxorubicin in culture medium, proteins in the culture medium were precipitated with 10% trichloroacetic acid, and the supernatant obtained after centrifugation at 7000*g* for 10 minutes was analyzed by HPLC.

The reversed-phase isocratic HPLC analysis was performed using a Pecosphere C18 column and a mobile phase of 30% acetonitrile in 20 mM potassium phosphate (pH 3.0), at a flow rate of 0.8 mL/min. Doxorubicin and epirubicin were detected with the use of a scanning fluorescence detector. The excitation and emission wavelengths were 480 nm and 550 nm, respectively. The retention time was approximately 7 minutes for doxorubicin and approximately 9 minutes for epirubicin. Standard curves were linear within the range of 1 ng/mL to 100 ng/mL (ie, 0.002 μM to 0.17 μM). Samples of culture medium containing high doxorubicin concentrations were diluted as needed.

Microscopic evaluation of doxorubicin penetration and distribution in tumors.

Spatial distribution of doxorubicin in tumor tissue was visualized using fluorescence microscopy. Histocultures were washed twice by dipping them in ice-cold drug-free medium, blot-drying, mounting on cryostat chucks with embedding matrix, placing them in a cryostat at -20°C, and cutting them into 10-μm sections. Sections were thaw-mounted on glass microscope slides and heat-fixed on a slide warmer for 15 minutes at 30°C. Slides were then covered with a coverslip, sealed

with rubber cement, and evaluated using fluorescence microscopy with excitation and emission wavelengths of 546 nm and 565 nm, respectively. The captured fluorescence images were analyzed using Optimas image analysis software (Silver spring, MD). At least 3 readings were obtained for each data point.

To establish the standard curves (each curve contained 6 data points) for measuring doxorubicin concentration in tissues by fluorescence microscopy, a doxorubicin solution (2 μL) was applied to microscopic sections of blank dog prostate tissue (10 μm thick), to cover a surface area of approximately 1.5 cm^2 to 2 cm^2. The average fluorescence intensity per area was measured and plotted against the applied doxorubicin concentrations to obtain the standard curves. When analyzing the doxorubicin in the actual samples, at least 3 sections were used per tumor and at least 3 tumors were used per time point.

Cell density

After tissue slides had been analyzed by fluorescence microscopy, the coverslips were removed and the slides stained with hematoxylin and eosin. The histologic images were captured using light microscopy and the cell densities were quantified using the Optimas software. Only cells with a diameter larger than 4 μm were counted.

Data analysis

Differences in mean values between groups were analyzed using the Student *t* test by SAS (Cary, NC).

RESULTS

Accumulation and retention of doxorubicin in CWR22 tumor histocultures

The accumulation and retention of doxorubicin in tumors was measured using HPLC, which specifically detects unchanged doxorubicin. The doxorubicin concentrations in the CWR22 tumor increased with time and reached plateau levels between 48 and 96 hours (Figure 1, Table 1). The maximum drug concentration in tumors increased with the initial drug concentration in the culture medium; the ratio of the maximal tumor concentration to the final concentration in the medium was about 100. The fractions of drug concentration remaining in tumors after 24 and 48 hours were about 60% and 40%, respectively. The high drug accumulation in the tumors and the slow drug release from the tumors are likely the result of the drug binding to intracellular macromolecules (8-9).

Similarities and differences in doxorubicin penetration and accumulation in patient and PC3 xenograft tumors

We compared the uptake of doxorubicin in patient and xenograft tumors. These tumors displayed different tissue composition and structure, which, as shown following, are important determinants of drug penetration. Because of the slow growth of the CWR22 tumor (1 g in ~7 weeks), the subsequent studies used the more rapidly growing PC3 tumor (1 g in ~4 weeks). Fluorescence microscopy was used

Table 1. Uptake, Accumulation, and Retention of Doxorubicin in CWR22 Tumor*

Initial concentration in culture medium (μM)	Final concentration in culture medium (μM)†	Maximum tumor concentration (μM) †	Tumor-to-medium concentration ratio at 96 hr†	Retention at 24 hr post treatment (%)†	Retention at 48 hr post treatment (%)†
0.02	0.008 ± 0.003	0.79 ± 0.15	94 ± 18	68 ± 12	30 ± 9
0.06	0.025 ± 0.009	2.87 ± 0.51	112 ± 20	57 ± 12	31 ± 6
0.12	0.055 ± 0.009	5.85 ± 0.80	105 ± 14	53 ± 23	45 ± 14
0.5	0.181 ± 0.084	21.6 ± 4.5	119 ± 25	56 ± 11	35 ± 9
2	0.888 ± 0.150	101 ± 12	114 ± 14	65 ± 8	52 ± 6
10	5.44 ± 0.53	458 ± 91	84 ± 11	68 ± 12	55 ± 8

*Drug uptake and accumulation were determined by measuring the drug concentration in CWR22 tumor histocultures treated with doxorubicin for 96 hours. In the drug retention study, the drug-containing medium was replaced with drug-free medium, and the fraction of drug retained in the tumor was expressed as a fraction of the concentration at the end of the 96-hour treatment.

†Mean ± standard deviation of 5 to 7 experiments.

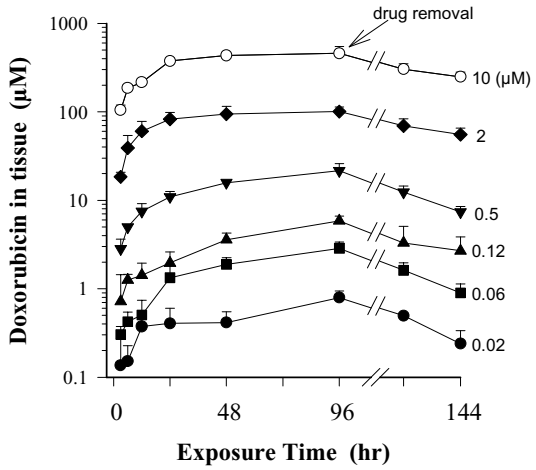

Figure 1. Kinetics of uptake, accumulation, and retention of doxorubicin in CWR22 xenograft tumor. Histocultures of the CWR22 tumor were treated with doxorubicin at the indicated initial extracellular concentrations. The drug-containing medium was replaced with drug-free medium at 96 hours.

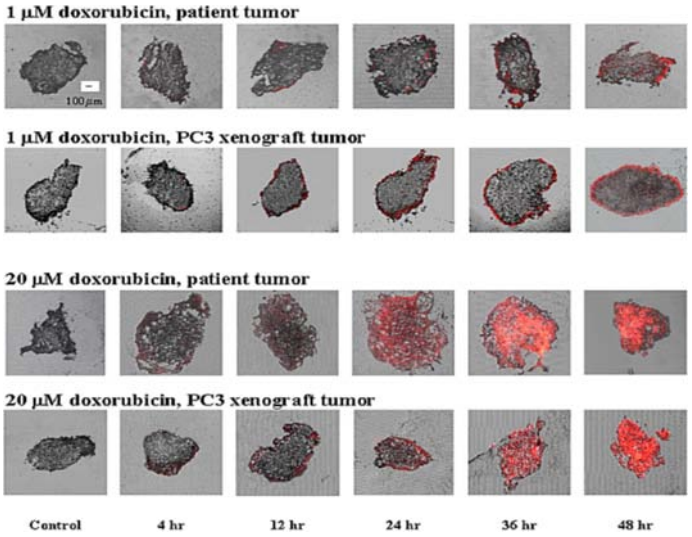

Figure 2. Doxorubicin penetration in tumor histocultures: spatial relationship with tumor cell distribution. Tumor histocultures were treated with 1 μM and 20 μM doxorubicin. Drug distribution was monitored by fluorescence microscopy as described in the Methods section. Magnification X 40.

to study the doxorubicin penetration and the intratumor concentration gradient as a function of the depth of drug penetration. Because the HPLC analysis of tumor homogenates did not detect the presence of doxorubicin metabolites (data not shown), the fluorescence intensity represented unchanged doxorubicin. The average width of the cross section of the histocultures was between 600μm and 800 μm, or about 60 to 100 cell-layers thick. We measured the drug penetration from the periphery (25 μm, referred to as periphery of tumor) to 325 μm, which represents the central region of the tumor (referred to as center of tumor).

Figure 2 shows the fluorescence micrographs. For both patient and xenograft tumors, drug accumulation in the periphery and the center of a tumor increased with time. At the lower concentration (ie, 1 μM), doxorubicin remained in the periphery of both patient and xenograft tumors at 72 hours. At higher concentrations (ie, 5 μM or 20 μM), doxorubicin was initially confined to the periphery (12 hours for patient tumors and 24 hours for xenograft tumors), followed by an abruptly enhanced drug penetration such that even distribution in histocultures was attained shortly after (24 hours for patients tumors and 36 hours for xenograft tumors).

Figure 3 shows the doxorubicin concentrations as a function of time and drug concentration. The data are presented as tumor-to-medium concentration ratios to standardize for the time-dependent changes in the extracellular drug concentration. Hence, a constant tumor-to-medium concentration ratio across the tumor indicates the attainment of equilibrium. Conversely, a declining concentration ratio from the periphery to the center indicates that the equilibrium was not achieved. For both patient and xenograft tumors, the concentration gradient from the periphery to the center of a tumor decreased with increasing treatment time and with increasing drug concentration. The periphery-to-center concentration gradient was the highest at the lowest initial extracellular concentration of 1 μM and decreased with increasing extracellular concentration, resulting in concentrations in the center being approximately equal to the

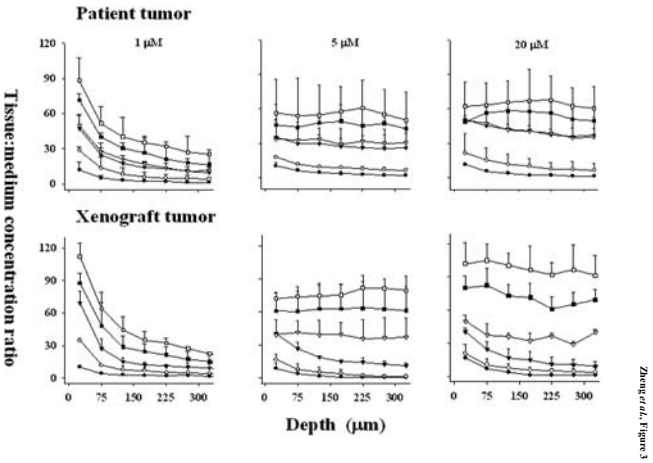

Figure 3. Concentration gradient of doxorubicin in solid tumors is dependent on treatment duration and initial extracellular drug concentration. Tumor histocultures were treated with 1 (left panels), 5 (middle panels), and 20 (right) μM of doxorubicin for 4(●), 12(○), 24(▼), 36(▽), 48(■) and 72(□) hours. The fluorescence intensity at different regions of the tumor, starting from the perimeter of the tumor, was measured and converted to doxorubicin concentration. Drug concentrations are represented as tumor-to-medium concentration ratios to standardize for differences in the drug concentration in the culture medium at different time points and different initial extracellular concentrations. Top panels: patient tumors. Bottom panels: PC3 xenograft tumor. Mean ± standard deviation of 3 tumors.

concentrations in the periphery after treatment with 5 μM and 20 μM for at least 24 hours. Compared to the patient tumors, the xenograft tumor showed greater periphery-to-center concentration gradients-as indicated by the steeper concentration decline over tissue depth-for all 3 initial extracellular concentrations. The time required to reduce the periphery-to-center concentration gradient to 0, after treatment with 5 μM and 20 μM doxorubicin, was shorter in the patient tumors than in the xenograft tumor (ie, 24 hours versus 36 hours). The differences between patient and xenograft tumors, as shown following, result from the differences in tissue composition and structure.

Effect of tumor cell density on doxorubicin penetration and accumulation in tumor

The previous data indicate a delay in doxorubicin penetration to the center of the tumor; and a longer delay in xenograft tumors than in patient tumors. We have shown that, for paclitaxel, this delay is not the result of drug diffusion from culture medium to histocultures, but is the result of a high tumor-cell density (14); a higher cell density corresponds with a smaller fraction of interstitial space. This results in increased tortuosity of the interstitial diffusion channels and slower drug diffusion (26,27). Similar findings were observed in the present study. Figure 4 shows that the xenograft tumor contained more tightly packed tumor cells, fewer stromal cells, and less interstitial space compared to patient tumors, which is consistent with the longer delay in doxorubicin penetration in xenograft tumors. The cell density in untreated histocultures of xenograft tumors was significantly higher than the density in patient tumors (2418 ± 66 versus 1864 ± 25 cells/mm^2, $P < .05$).

In both xenograft and patient tumors, we observed a higher fluorescence intensity in cells compared with interstitial space. This observation, together with the observation of the extensive doxorubicin accumulation in tumor cells (ie, 100X the extracellular concentration; see Table 1), suggest the difference in cell density in patient and xenograft tumors as the cause of the difference in their drug accumulation. The magnitude of the difference in cell density between the xenograft and patient tumors (23%) is within the range of the difference of drug accumulation between these tumors (13% to 33%).

Effect of drug-induced cell death on doxorubicin penetration in tumor

The abrupt change in the rate of doxorubicin penetration to the center of a tumor was observed only at the higher concentrations of 5 μM and 20 μM and not at 1 μM (Figures 2 and 3). The 5-μM and 20-μM concentrations were near to or exceeded the drug concentration required to produce 50% cell death for a 96-hour treatment (23), whereas 1 μM was below this concentration. Figure 4 shows the reduction of tumor cell density over time after treatment with 20 μM doxorubicin, although no change was observed after treatment with 1 μM doxorubicin. This suggests that the abrupt change in drug penetration that occurred only after treatment with high drug concentrations is the result of drug-induced cell death and reduction of cell density. This is further supported by the inverse correlation between the average tumor concentration and the tumor cell density in the periphery of the xenograft tumor after treatment with 20 μM doxorubicin (Figure 5).

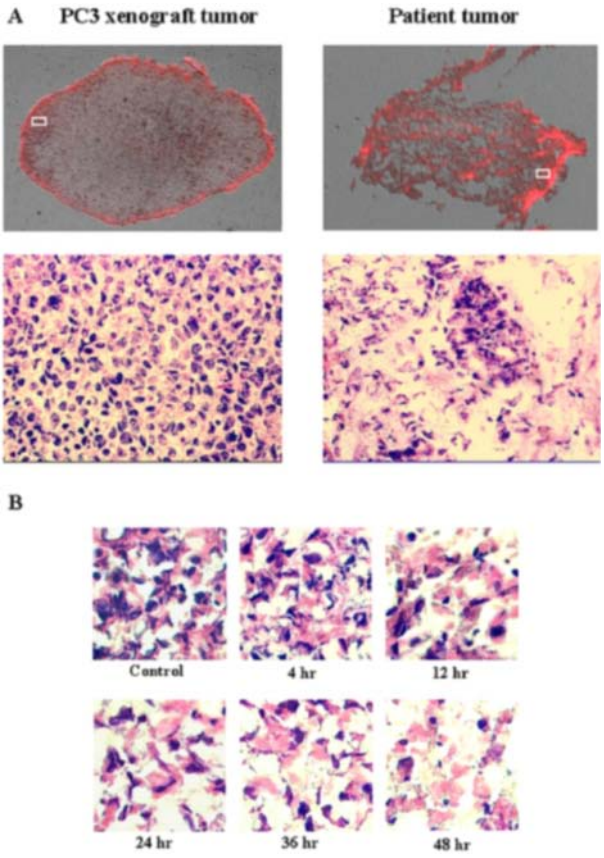

Figure 4. Effect of cell density on doxorubicin penetration. (A) Tumors were treated with 1 μM doxorubicin for 72 hours. Upper panels: fluorescence microscopic images; magnification X 40. Bottom panel: enlargement of the indicated boxed region from the upper panel; this demonstrates the difference in the tissue composition in the PC3 xenograft (left) and the patient (right) tumor; magnification X 400. (B) Tumor cell density decreased with increasing treatment time in the PC3 xenograft tumor treated with 20 mM doxorubicin. Histologic images (stained with hematoxylin and eosin); magnification X 400.

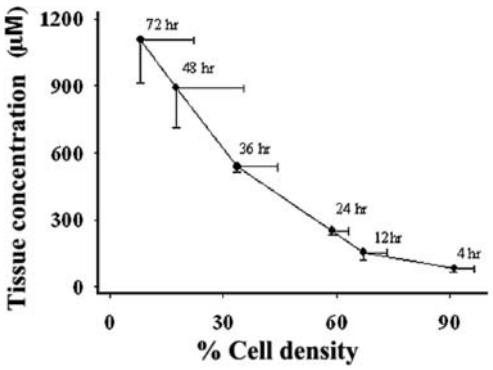

Figure 5. Effect of cell density on doxorubicin accumulation. Histocultures of the PC3 xenograft tumor were treated with 20 μM of doxorubicin. The cell density in the periphery (100 μm from the perimeter) was measured, and expressed as the ratio between the treated samples and the untreated controls. Mean ± standard deviation of 3 tumors.

DISCUSSION

This study's results indicate that the rate of doxorubicin penetration to the center of the tumor depends on initial extracellular drug concentration, treatment time, and tumor type (ie, tissue composition). Drug penetration was faster at higher concentrations, and the effect of concentration on the penetration rate was more significant in tumors with high tumor-cell density than in tumors with low density. A minimum treatment time of 24 to 36 hours was required for doxorubicin to penetrate a depth of 300 μm. Our results also indicate that the extent of maximal doxorubicin accumulation in tumor cells in a solid tumor depends on initial extracellular drug concentration, treatment time, tumor type, and tumor cell density.

Our finding that the penetration of doxorubicin in a solid tumor is confined to the periphery in the first 12 hours is consistent with the findings in tumor spheroids (7-9). The slow penetration of doxorubicin is considered characteristic for high molecular weight molecules as a result of its extensive binding to proteins (1,9). The finding that a high tumor-cell density reduced doxorubicin penetration in a solid tumor is consistent with an earlier finding that coating of Teflon membranes with a multilayer of tumor cells (2-4 ± 106 cells, 200 μm thick) reduces the transmembrane transport of doxorubicin by more than 90% (11).

The two mechanisms often implicated in the slow penetration of drugs into solids tumors are the impeded influx resulting from high oncotic pressure (28) or the drug efflux by the *mdr1* p-glycoprotein (29). But neither of these mechanisms could be the

cause of the slow doxorubicin penetration in the PC3 histocultures because PC3 cells do not express Pgp (30,31) and because histocultures lack the capillary blood flow needed to supply the oncotic pressure.

When compared with patient tumors, the higher density of epithelial cancer cells in xenograft tumors correlates with a slower drug penetration rate and a higher drug accumulation. These data suggest cellularity as a major determinant of the rate and extent of doxorubicin penetration and accumulation in solid tumors. Qualitatively, these findings are identical to our previous observations on the paclitaxel penetration and accumulation in solid tumors (14). As shown earlier for paclitaxel, apoptosis is required for enhanced drug penetration. Under conditions in which either insufficient drug concentration or insufficient time for apoptosis occurs, no enhancement in paclitaxel penetration was observed (32). Hence, enhanced drug penetration in solid tumors caused by drug-induced cell death appears a common phenomenon for at least 2 highly protein-bound drugs (ie, paclitaxel and doxorubicin). Our observations further demonstrate an interesting new concept in the relationship between drug delivery and drug effect. In addition to the general belief that drug delivery to tumor cells determines the antitumor activity, our finding indicates that the pharmacological effect of a drug can modify its delivery.

The finding that drug-induced apoptosis resulted in enhanced drug penetration in solid tumors may have clinical implications. We recently completed a study investigating the effect of dosing regimens on delivery of paclitaxel to solid tumors. The results showed that an initial apoptosis-inducing loading dose, followed by a second dose administered when apoptosis had occurred, resulted in a 50% higher drug concentration in tumors as compared with other treatment schedules where either the dose intensity was not sufficient to induce apoptosis or the dosing intervals were not sufficiently long for apoptosis to take place (32). It is noted that the doxorubicin concentrations used to induce apoptosis in the PC3 histocultures exceeded the clinically achievable concentrations (i.e., 5 μM versus 200 nM) (33-35). However, the 200 nM doxorubicin concentration is sufficient to induce cell death in histocultures of patient tumors (23). Additional in vivo studies, such as those described for paclitaxel (32), are needed to determine whether the tumor delivery of doxorubicin can be enhanced by manipulating the dosing schedule.

In summary, our results indicate drug-induced cell death as a key determinant of the rate and extent of doxorubicin penetration in solid tumors. The delivery of doxorubicin to cells in a solid tumor is a dynamic process determined by both the drug concentration and the treatment duration and the usual processes involved in drug transport (ie, distribution through vascular space, transport across microvessel walls, and diffusion through interstitial space in tumor tissue). That the pharmacological effects of doxorubicin affect its delivery will need to be taken into consideration when designing treatment schedules to maximize the drug delivery to the hard-to-reach tumor cells distant from the vasculature or from a regional delivery site. For example, a treatment schedule to include a pretreatment to induce cell death may enhance drug delivery to such sites.

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