Limited Penetration of Anticancer Drugs through Tumor Tissue: A Potential Cause of Resistance of Solid Tumors to Chemotherapy[1]

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ABSTRACT

Purpose: **Potential causes of drug resistance in solid tumors include genetically determined factors expressed in individual cells and those related to the solid tumor environment. Important among the latter is the requirement for drugs to penetrate into tumor tissue and to achieve a lethal concentration in all of the tumor cells. The present study was designed to characterize further the multicellular layer (MCL) method for studying drug penetration through tumor tissue and to provide information about tissue penetration for drugs used commonly in the treatment of human cancer.**

Experimental Design: **EMT-6 mouse mammary and MGH-U1 human bladder cancer cells were grown on collagen-coated semiporous Teflon membranes to form MCLs ~200 μm thick. The properties of MCLs were compared with those of tumors grown in mice from the same cells. The penetration of drugs through the MCL was evaluated by using radiolabeled drugs or analytical methods.**

Results: **The MCL developed an extracellular matrix containing both laminin and collagen, although there were some differences in expression of extracellular matrix proteins. Electron microscopy showed rare desmosomes in both MCL and tumors. The penetration of cisplatin, etoposide, gemcitabine, paclitaxel, and vinblastine through tissue in the MCL was slow compared with penetration through the Teflon support membrane alone.**

Conclusions: **Our results suggest limited ability of anticancer drugs to reach tumor cells that are distant from blood vessels. The limited penetration of anticancer drugs through tumor tissue may be an important cause of clinical resistance of solid tumors to chemotherapy.**

INTRODUCTION

Most solid tumors are rather resistant to chemotherapy. There is a substantial volume of literature that has provided information about a wide variety of mechanisms that can lead to resistance of individual cancer cells. These mechanisms include the presence of a range of drug export pumps (such as P-glycoprotein or multidrug resistance-associated protein), changes in the expression of topoisomerases, and alterations in metabolic pathways that influence drug metabolism, DNA repair, or apoptosis. A smaller number of publications has drawn attention to causes of drug resistance that depend specifically on the microenvironment (1, 2) and/or on problems relating to the delivery of anticancer drugs to the tumor cells (3–13). These mechanisms may have equal or greater influence on clinical resistance of tumors as compared with cellular factors and may be amenable to strategies that would render tumors more sensitive to therapy.

Anticancer drugs gain access to solid tumors via the blood supply and must penetrate through the extravascular space to reach all of the cancer cells in sufficient concentration to cause lethal toxicity. Factors that influence the distribution of drugs in tumors include delivery by the vascular system, the rate of diffusion through tissue, drug metabolism, and binding to tissue components (8, 9, 14, 15). Blood flow in tumors is often irregular, and the intercapillary distance may be relatively large compared with normal tissue (16, 17). Thus, tissue penetration of anticancer drugs is likely to be more limited in tumors than in normal tissues.

Model systems evaluating the distribution of drugs in spheroids, or the assessment of cell survival after use of Hoechst 33342 fluorescence to separate cells on the basis of their distance from the functional blood vessels in tumors, have provided measures of drug penetration (3–7). Recently, Hicks *et al.* (14) and Cowan *et al*. (18) have developed a simpler *in vitro* model that allows for the direct assessment of the penetration of chemotherapeutic agents through solid tissue. Tumor cells are grown on microporous Teflon membranes as MCLs[3] that have tumor-like physiology. We have used this method to demonstrate the limited penetration of doxorubicin, 5-FU, methotrexate, and mitoxantrone through solid tissue (10) and to study some of the factors that influence penetration of doxorubicin and methotrexate (12, 13). Variants of this technique have also been established in other laboratories (19, 20). This relatively new method allows the direct characterization of the ability of anticancer drugs to penetrate tissue, a property of comparable

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[3] The abbreviations used are: MCL, multicellular layer; 5-FU, 5-fluorouracil; ECM, extracellular matrix.

importance to cellular causes of drug sensitivity in determining the likely outcome of chemotherapy for solid tumors.

The goals of the present study are to provide information about the relevance of the MCL model for studies of drug penetration *in vivo* and to provide baseline information about the tissue penetration of other anticancer drugs that are in common use to treat human cancer. We therefore compare properties of MCLs with those of solid tumors derived from the same cells. We then investigate the penetration through MCLs of the following drugs that are in common use to treat human solid tumors: cisplatin, etoposide, gemcitabine, paclitaxel, and vinblastine. Our hypotheses are that penetration of some of these drugs through tissue will be slow, and that slow tissue penetration will contribute to clinical resistance.

MATERIALS AND METHODS

Cells and Tumors. Experiments were performed using the mouse mammary sarcoma cell line, EMT-6 (obtained originally from Dr. R. Sutherland, University of Rochester, Rochester, NY) and the human bladder carcinoma cell line, MGH-U1 (obtained originally from Dr. G. Prout, Massachusetts General Hospital, Boston, MA). Cells were grown as monolayers in α-MEM (Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum (Cansera, Toronto, Ontario, Canada) at 37°C in a humidified atmosphere of 95% air plus 5% CO_2. Cells were re-established from frozen stock every 3 months. *Mycoplasma* tests were consistently negative. All experiments were performed using exponentially growing cells.

MGH-U1 or EMT-6 tumors were generated by i.m. injection of 2–5 $\times$ 10^5 tumor cells into the left hind leg of inbred female Swiss nude and BALB/c BYJ (Jackson Laboratories, Bar Harbor, ME) mice, respectively. Tumors were used when they had grown to a mean diameter of ~1 cm, which required ~7 days for the murine EMT-6 tumors and 12 days for the MGH-U1 xenografts.

Growth of MCLs. Exponentially growing cells (2 $\times$ 10^5) were seeded on a microporous Teflon membrane culture plate insert that had been coated previously with collagen (Millipore, Bedford, MA). Cells were allowed to attach for 4–24 h, and the membranes were then submerged in a large volume of stirred culture medium and allowed to grow for 4 days (for EMT-6 cells) or 6 days (for MGH-U1 cells). The growth of the MCL was studied by selecting and trypsinizing MCLs at various times after their initiation, and cells were counted using a Coulter counter.

Characterization of MCLs. Some MCLs were fixed in formalin, embedded in paraffin, sectioned, and stained with H&E. Other MCLs were stained with Masson's trichrome for evaluation of the ECM or were subjected to immunofluorescence staining for matrix proteins. The antisera used were rabbit antilaminin (Sigma Chemical Co.), rabbit anticollagen type I (Chemicon International, Inc., Temecula, CA), and mouse monoclonal anticollagen type IV (Dako Corp., Carpinteria, CA). Briefly, tissue sections were dewaxed in toluene and rehydrated through graded alcohol to water. Endogenous peroxidase activity was blocked by a 3% hydrogen peroxide solution. After performing antigen retrieval and pretreatment with pepsin digestion, slides were incubated with the primary antibody for 1 h at room temperature. After being washed in PBS, secondary incubations were carried out with biotin antirabbit (Vector Laboratories, Burlingame, CA) or antimouse (Signet Pathology Systems, Inc., Dedham, MA) IgG, followed with streptavidin-horseradish peroxidase (Signet Pathology Systems, Inc.) for 30 min. Immunoreactivity was demonstrated by incubation in 3-amino-9-ethylcarbazol. Slides were counterstained in hematoxylin and mounted with Crystal Mount.

Tumors and MCLs were fixed for electron microscopy in universal fixative (1% glutaraldehyde, 4% formaldehyde in 0.7 M phosphate buffer). They were then postfixed, dehydrated, and embedded in Epon. Thin sections were stained with uranyl and lead acetate and examined with a JEOL 1200 transmission electron microscope.

Anticancer Drugs. [^{3}H]Etoposide (specific activity, 618 mCi/mmol), [5-^{3}H]gemcitabine (specific activity, 14 Ci/mmol), [^{3}H]paclitaxel (specific activity, 5 Ci/mmol), and [^{3}H]vinblastine (specific activity, 5 Ci/mmol) were purchased from Moravek Biochemicals (Brea, CA). [^{14}C]Doxorubicin (specific activity, 54 mCi/mmol) was purchased from Amersham Life Sciences (Buckinghamshire, United Kingdom). [^{3}H]5-FU (specific activity, 5 Ci/mmol), [^{3}H]sucrose (specific activity, 10.2 Ci/mmol), and [^{14}C]sucrose (specific activity, 600 mCi/mmol) were obtained from DuPont NEN (Billerica, MA). Unlabeled cisplatin, gemcitabine, paclitaxel, vinblastine, 5-FU, and dexamethasone were obtained as their clinical formulations. Cisplatin and vinblastine were obtained from Faulding Canada (Vandreuil, Quebec, Canada), gemcitabine was obtained from Eli Lilly Canada (Toronto, Ontario, Canada), paclitaxel was obtained from Bristol-Myers Squibb Canada (Montreal, Quebec, Canada), 5-FU was obtained from Pharmacia and Upjohn (Uppsala, Sweden), and dexamethasone was obtained from Sabex (Boucherville, Quebec, Canada).

Penetration of Anticancer Drugs. All experiments were performed at 37°C in a humidified environment of 95% air and 5% CO_2. During experiments, MCL inserts were floated on a pool of 18 ml of fresh medium with fetal bovine serum (Fig. 1). The penetration of anticancer drugs through the MCL was studied by adding the drug of interest to the MCL insert (compartment 1). At various times after adding the drug, 250-μl samples were withdrawn from compartment 2 and analyzed for drug content. Penetration of each drug through a cell-free insert was used as a control. All drug solutions contained 1% agar (except as otherwise stated) to prevent convective movement in compartment 1. A randomly selected MCL was trypsinized and the total number of cells was counted. MCLs were used only if the cell number was in the range of 2.5–4 $\times$ 10^6. In all experiments (except in the studies of cisplatin), penetration of 1.4 μM [^{14}C]sucrose or 820 nM [^{3}H]sucrose was evaluated as an internal standard to ensure similar thickness of the MCL. Sucrose is excluded from cells and is not metabolized; therefore, its penetration depends only on the thickness of the MCL for any particular cell line.

Unless otherwise stated, the initial concentrations of drugs in compartment 1 were as follows: 20 μM cisplatin, 20 μM [^{3}H]etoposide, 100 μM [^{3}H]gemcitabine, 25 μM [^{3}H]paclitaxel, 11 μM [^{3}H]vinblastine, 10 μM [^{14}C]doxorubicin, or 78 μM [^{3}H]5-FU. These concentrations are close to those that can be achieved in serum after administration *in vivo* and allow the

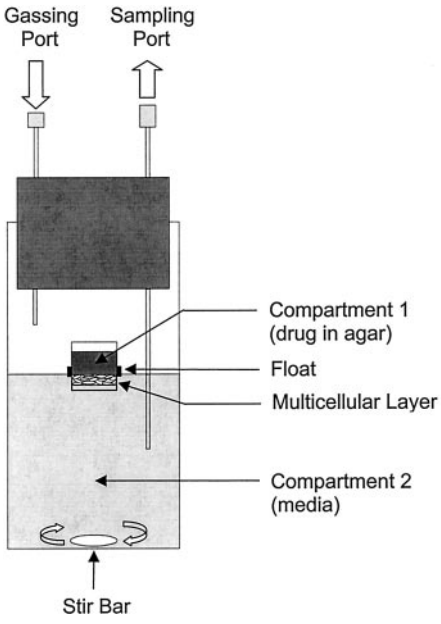

Fig. 1 Experimental system used to measure penetration of anticancer drugs through the MCL. The MCL insert is floated in media. Drugs at selected concentrations are added to compartment 1 in 1% agar. Samples are withdrawn through the sampling port from compartment 2 at different times. Vials are gassed with a mixture of 95% air and 5% CO_2.

sensitive detection of the drugs in compartment 2 of Fig. 1, on the other side of the MCL. Cisplatin concentration was assessed with a Perkin-Elmer model 1100 flameless atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT) as described previously (21). The penetration of radiolabeled drugs was measured by scintillation counting. It is recognized that the measurement of radioactivity in compartment 2 sets an upper limit on the penetration of the parent compound because some of the measured radioactivity might be associated with metabolites.

RESULTS

Characterization of MCLs. To determine whether MCLs provide appropriate models for tissue in solid tumors, we studied their growth kinetics and compared some of the properties of MCLs with those of transplanted tumors generated from the same cell lines.

The number of cells in MCLs derived from MGH-U1 cells increased exponentially over 6 days from $\sim 2 \times 10^5$ to $\sim 3–4 \times 10^6$, and by this time the MCL had a thickness of ~ 200 μm. MCLs derived from the EMT-6 cell line grew more rapidly to reach a similar size in 4 days. The central layers of the MCLs showed early necrosis at this thickness.

The composition of the ECM in the MCL was compared with that of tumors grown from the same cell lines by using immunohistochemistry (Fig. 2). Tumors and MCLs had a similar histological appearance when stained with either H&E or Masson's trichrome. By immunohistochemistry, MCLs grown from EMT-6 cells had qualitatively similar levels of laminin and more intense staining for collagen I as compared with EMT-6 tumors. In MCLs derived from MGH-U1 cells, there was similar staining for laminin but less intense staining for collagen I than in MGH-U1 xenografts, whereas collagen IV was detected in the tumors but not the MCLs (Fig. 2).

Epithelial tissues and tumors usually contain regions of close cellular contact such as desmosomes and gap junctions. We were able to identify desmosomes in electron micrographs of tumors and MCLs derived from either MGH-U1 or EMT-6 cells, although they were rare in both MCLs and the transplanted tumors.

Drug Penetration through MCLs. In these experiments, penetration was characterized as the ratio of radioactivity (or measured drug concentration) in compartment 2 (Fig. 1) to the calculated value when the drug had reached equilibrium (C_{infinity}).

The penetration of gemcitabine through MCLs derived from MGH-U1 cells was used to characterize some general properties of the experimental system (Fig. 3). The influence of agar, to which drug is added to compartment 1 (Fig. 1) to prevent variable effects attributable to convection, was evaluated in experiments illustrated in Fig. 3*A*. Here the penetration of gemcitabine through the cell-free insert was studied when added directly in agar or with an intervening layer of medium in agar (without drug). There is a short delay of penetration representing the retarding effect of the agar. We also evaluated the penetration of gemcitabine in single layers of agar in the range of concentration of 0.25–2% and found no difference in penetration (data not shown). In Fig. 3*B*, the penetration of gemcitabine through MCLs of different thickness is shown. As the number of cells and thickness of the MCL increase, penetration of gemcitabine decreases. In Fig. 3*C*, the effect of the concentration of gemcitabine (50–400 μM) has been evaluated; the relative drug penetration through the MCL, expressed as a ratio of total drug added to the system, was independent of the concentration of gemcitabine added to compartment 1.

Fig. 4 shows the relative penetration of etoposide, paclitaxel, vinblastine, and cisplatin or gemcitabine through MCLs derived from MGH-U1 cells (Fig. 4*A*) and EMT-6 cells (Fig. 4*B*) as compared with penetration of the same drugs through the Teflon membrane alone. In MCLs derived from MGH-U1 cells, vinblastine showed a slower rate of penetration through MCLs (~20%) than cisplatin, paclitaxel, and etoposide (30–50%). The rate of penetration of vinblastine and paclitaxel (30–40%) was also slower than that of gemcitabine or etoposide (~50%) through MCLs derived from EMT6 cells. Note that for each of these drugs, the MCL provides a barrier such that the rate of penetration is at most ~50% of that through the Teflon membrane alone.

DISCUSSION

Most studies of the causes of resistance of solid tumors to chemotherapy have concentrated on molecular and cellular mechanisms that lead to resistance of the constituent cells. Such mechanisms undoubtedly contribute to the resistance of solid tumors to chemotherapy, but mechanisms related to the microenvironment will also influence clinical sensitivity. Some types of resistance to anticancer drugs are expressed only when cells are grown in close contact (1, 2), and a requirement for effective chemotherapy is that drugs penetrate to achieve a lethal con-

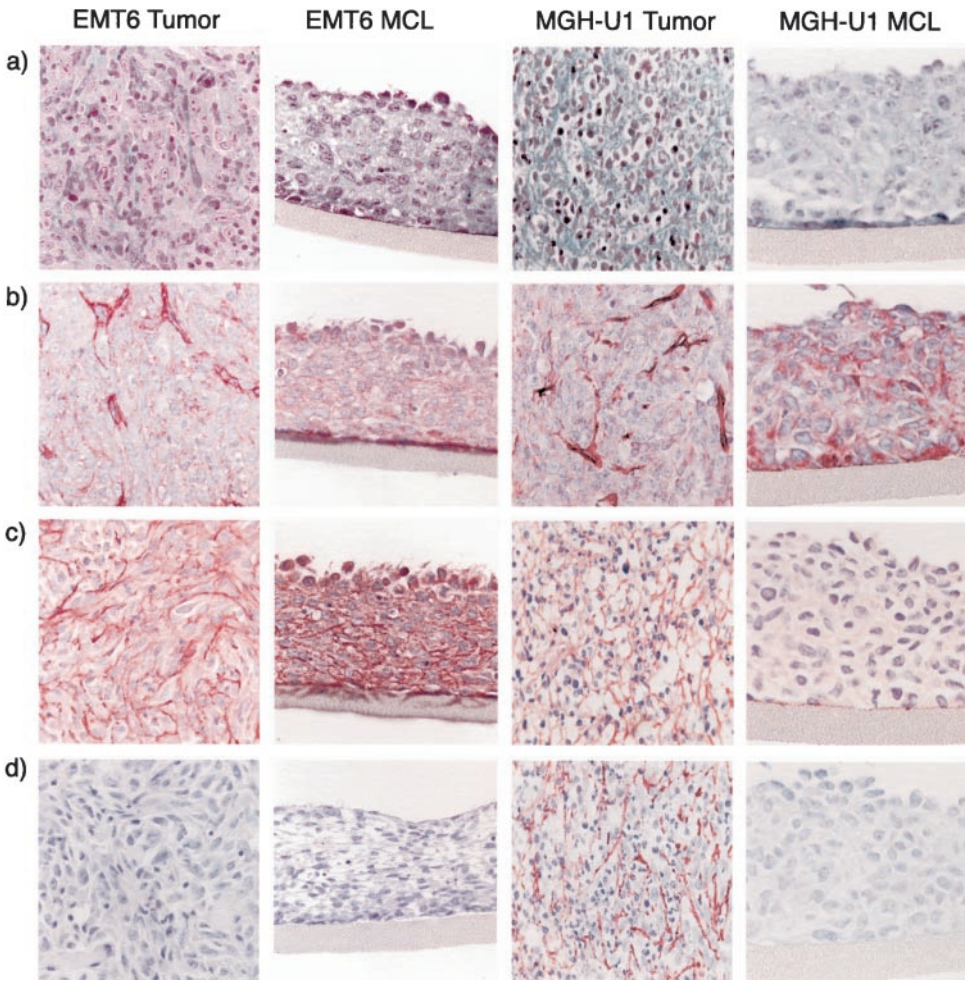

Fig. 2 Demonstration of an ECM in tumors and MCLs derived from EMT-6 and MGH-U1 cells. The sections were stained by Masson's trichrome (*a*) and by indirect immunofluorescence for laminin (*b*), collagen type I (*c*), and collagen type IV (*d*), respectively. Antisera used were rabbit antilaminin, rabbit anticollagen type I, and mouse monoclonal anticollagen type IV. The last of these antibodies recognizes only human collagen type IV, and murine EMT-6 cells (*d*) are included as a concurrent negative control stained with an irrelevant antibody.

centration in all of the target cancer cells. Earlier studies of the distribution of radiolabeled or fluorescent anticancer drugs in spheroids had suggested poor penetration (3, 4), but these methods were subject to potential artifact during tissue preparation. The use of Hoechst 33342 to establish a gradient of fluorescence from the surface of spheroids or from functional blood vessels in experimental tumors, followed by drug treatment and cell sorting, also showed poorer drug activity for less fluorescent cells, consistent with poor penetration of drugs into tissue (5–7). This method gives very useful information but is quite complex and indirect. The recent development of the MCL method (10, 12–15, 18–20) has provided a simple and direct model for studying the penetration of anticancer drugs through tissue derived from tumor cells. The goals of the present study were to provide information about the relevance of the MCL model for studies of drug penetration *in vivo* and to provide baseline information about the tissue penetration of some anticancer drugs in common use that have not been evaluated previously in this way.

We undertook studies to compare the properties of MCLs and of transplanted tumors generated in mice from the same cells and to evaluate the relevance of the model for studying tumor tissue *in vivo*. Potential limitations of the MCL model include the requirement to add drugs in dilute agar to prevent convection and the additional barrier to penetration of the collagen-coated Teflon membrane that is required to support the MCL. Variants of the model have been used to study drug transfer between two completely stirred compartments on either side of the MCL (20). This has the advantage of allowing drug concentration to be measured (and varied) in the leaving compartment but does not reflect the situation in tumors where the capillary wall acts as a barrier to convection. Here we have shown that the agar in which the drug is added causes only a minor delay in drug penetration of the MCL, and that this delay is not sensitive to the concentration of agar that is used. The Teflon membrane does present an additional barrier to drug penetration, with drug concentration reaching ~60% of the equilibrium concentration after 6 h. A reasonable assumption is that for any given drug, the barrier to drug penetration posed by the MCL is additive to that posed by the Teflon membrane alone, although admittedly this is difficult to test experimentally.

We have shown the presence of an ECM in the MCL that contains both laminin and collagen (Fig. 2). There are, however, some differences in the relative expression of ECM proteins between the MCL and the corresponding tumors, and collagen

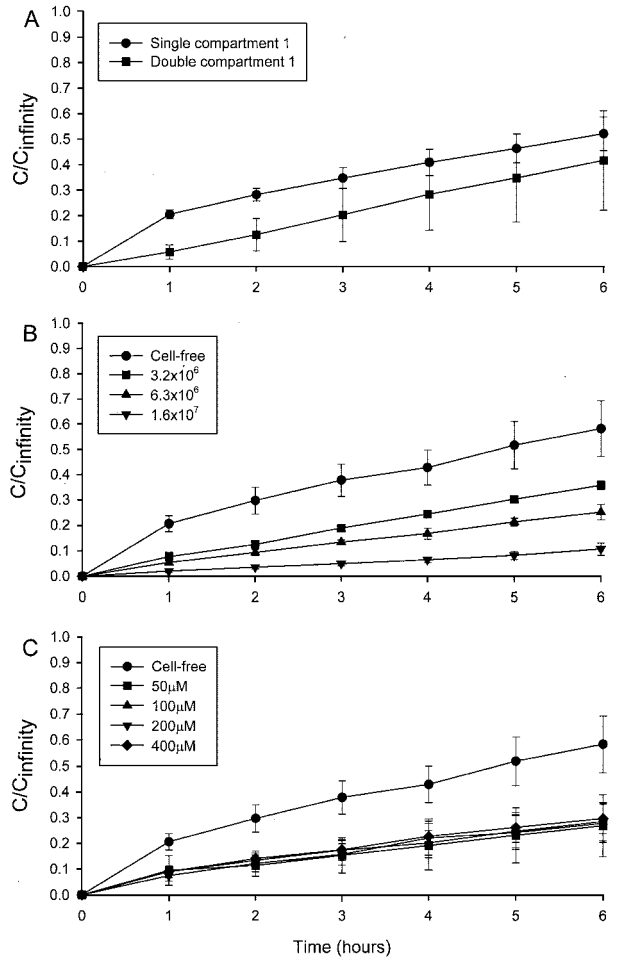

Fig. 3 Penetration of [^{3}H]gemcitabine as a function of time through MCLs derived from MGH-U1 cells. *Symbols* represent the concentration (*C*) in compartment 2 (as a ratio of the expected concentration at equilibrium, $C_{infinity}$). [^{3}H]Gemcitabine was added to compartment 1. The radioactivity in compartment 2 was measured by scintillation counting. *A*, penetration of gemcitabine in a cell-free system. ●, penetration of gemcitabine in a single layer of agar; ■, penetration of gemcitabine when added to a second layer of agar above a first layer that does not (initially) contain drug. *B*, penetration of gemcitabine through MCLs of different thicknesses. ●, penetration of gemcitabine in the cell-free system; *other symbols*, penetration of gemcitabine through MCLs containing different numbers of cells as indicated. *C* shows that there is no effect of different total concentrations of gemcitabine added to compartment 1 on the relative rate of penetration through the MCL. Data *points*, means of three or more experiments; *bars*, SE.

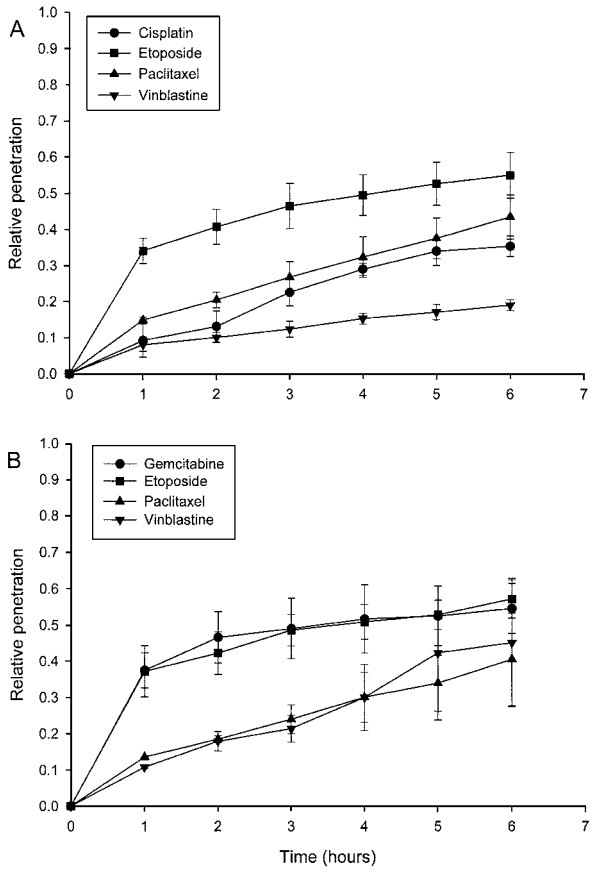

Fig. 4 Penetration of [^{3}H]etoposide, [^{3}H]paclitaxel, [^{3}H]vinblastine, cisplatin, and/or [^{3}H]gemcitabine through MCLs derived from MGH-U1 cells (*A*) and EMT-6 cells (*B*) as a function of time. *Symbols*, relative penetration of drug through the MCL as compared with penetration of the same drug through the Teflon membrane alone. The drug of interest was added to compartment 1. The drug concentration in compartment 2 was measured as described in the text. Data *points*, means of three or more experiments; *bars*, SE.

type IV was present in MGH-U1 tumors but not MCLs. Electron microscopy showed the presence of only rare desmosomes in both tumors and MCLs, and perhaps this is characteristic of tissue derived from tumor cell lines that have been maintained for long periods in culture. Previous studies from our laboratory have shown that the penetration through MCLs of doxorubicin and methotrexate is increased under conditions where uptake of the drugs into cells is inhibited: expression of P-glycoprotein in the cells of the MCLs for doxorubicin, acid conditions, or presence of folic acid for methotrexate (12, 13). Thus, penetration of drugs through tissue may be largely through the ECM. Future studies of the relationship between drug penetration and components of the ECM, the presence and number of tight junctions between cells, and other extracellular properties may provide important clues as to how drug penetration might be modified.

We found that the relative penetration of gemcitabine (expressed as a proportion of the initial concentration added to compartment 1 of Fig. 1) was independent of drug concentration but (not surprisingly) decreased with increasing thickness of the MCL (Fig. 3). This result suggests that drug penetration, and hence effectiveness, will be poor in tumors that have a large distance between patent blood vessels. Increasing the concentration of drugs in compartment 1 (and by extrapolation increasing drug dose *in vivo*) is likely to lead to a proportional increase in drug concentration throughout the tissue. However, most drugs are used at doses close to the maximum that is tolerated by normal tissues, and therefore a large increase in drug dose is unlikely to be possible.

The penetration of anticancer drugs through tissue depends on the effective diffusion coefficient, on metabolism of the drugs, and on binding in tissue. Hicks *et al.* (14) and Wilson and Hicks (15) have proposed a mathematical model for analyzing the time-dependent penetration of anticancer drugs through MCLs in terms of these factors. We have not applied this analysis to our data because different models lead to only small changes in the predicted penetration *versus* time curves, and the data are not precise enough to differentiate between the various mechanisms that lead to slow penetration of tissue. Investigation of such mechanisms will require definition of the profile of drug concentration across MCLs by autoradiography or other means and of the distribution of each drug and its metabolites in cellular compartments.

We have studied the penetration through MCLs of both older (cisplatin, etoposide, and vinblastine) and newer (gemcitabine and paclitaxel) drugs that are used in the treatment of solid tumors. Where available, we have used ^{3}H- or ^{14}C-radiolabeled drugs to study penetration, because assessment is then simple and accurate. We recognize, however, that some of the label might be associated with metabolic products rather than with the parent compound. Because we are detecting the total amount of label that has penetrated the MCL, some of which may be associated with metabolites, the method sets an upper limit for the rate of penetration of the parent compound through the MCL.

Limited tissue penetration appears to present a substantial barrier to the effectiveness of each of the agents that we have evaluated. Penetration was poorest for vinblastine (~20–40% of the penetration through the support membrane alone at 6 h), followed by cisplatin, paclitaxel, and gemcitabine, and best for etoposide (~50% of the rate of penetration through the support membrane alone at 6 h). The present data complement our previous study of the penetration of doxorubicin, 5-FU, methotrexate, and mitoxantrone through MCLs derived from human breast cancer MCF-7 cells (10). Because we have found minimal influence of cell type on drug penetration through MCLs, we have included a comparison of relative tissue penetration of each of these drugs in Fig. 5. The basic drugs doxorubicin and mitoxantrone have particularly poor tissue penetration, likely because of their sequestration in acidic endosomes of cells and their binding to DNA (22, 23).

For drugs with poor penetration of tissue, there is likely to be a limit on cell kill after a single administration, even if the constituent tumor cells are highly sensitive. Clinical responses do occur and are probably then attributable to killing of successive layers of cells during successive courses of treatment, a bit like peeling an onion (or an inside-out onion surrounding a tumor blood vessel). Strategies that might improve the cell kill attributable to limited penetration of tissue include the following:

(*a*) Modification of the factors that lead to poor penetration (*e.g.*, modification of the ECM to influence the diffusion coefficient or decreased binding or sequestration of drug in tissue compartments that are not contributing to its lethal action, such as endosomes).

(*b*) Delivery of drugs by continuous infusion to allow attainment of equilibrium concentration of drugs throughout tissue. In support of this concept, Durand (24) has shown that the doxorubicin concentration in deeper layers of spheroids increases markedly with chronic administration of drug at the spheroid surface. Slow penetration of tumor tissue could then lead to better drug retention in tumors. Although continuous infusion might require dose reduction to prevent increased toxicity to normal tissues, this strategy might improve therapeutic index by overcoming the relative disadvantage of tumors attributable to their poor vascular access.

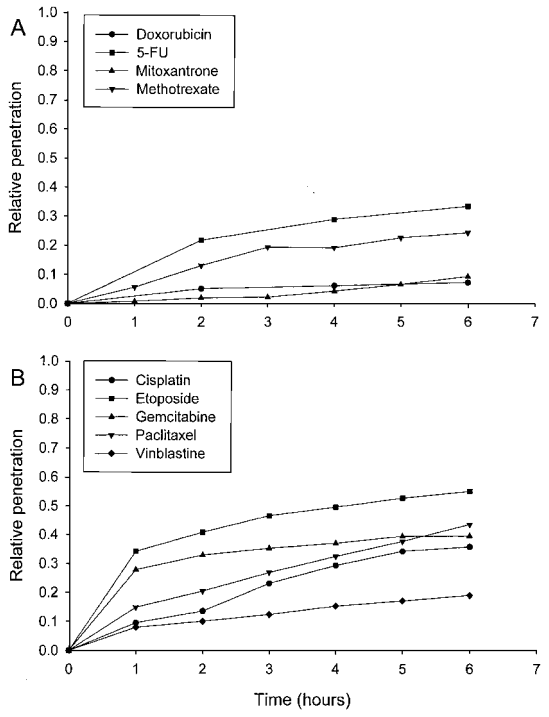

Fig. 5 Relative penetration of some commonly used anticancer drugs through MCLs derived from MCF-7 cells (*A*) and MGH-U1 cells (*B*). Error bars have been removed for clarity but are of similar size to those in Fig. 4.

(*c*) Modification of drug penetration by physical means, *e.g.*, by using hyperthermia or low-dose irradiation. MCLs provide a useful model for evaluating these strategies.

(*d*) Selective inhibition of repopulation of surviving tumor cells between courses of chemotherapy (25) to prevent regrowth from cells that are spared initially because of poor drug penetration (prevention of regrowth of the onion, using the above analogy). This might be achieved by using biological agents, such as inhibitors of growth factors that are selectively expressed on tumor cells.

In summary, the causes of drug resistance of human solid tumors are multifactorial. Although genetically determined causes of cellular resistance undoubtedly contribute to effective resistance of human tumors, they represent only one contributing mechanism. Here we provide evidence for a mechanism that may also have a profound effect on the outcome of chemotherapy—the limited penetration of anticancer drugs through tissue. Even a drug to which the constituent tumor cells are highly sensitive will have limited efficacy if it only reaches some of the target tumor cells in low concentration.

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REFERENCES

1. Teicher, B. A., Herman, T. S., Holden, S. A., Wang, Y. Y., Pfeffer, M. R., Crawford, J. W., and Frei, E. Tumor resistance to alkylating agents conferred by mechanisms operative only *in vivo*. Science (Wash. DC), *247:* 1457–1461, 1990.

2. Kerbel, R. S., Rak, J., Kobayashi, H., Man, M. S., St. Croix, B., and Granham, C. H. Multicellular resistance: a new paradigm to explain aspects of acquired drug resistance of solid tumors. Cold Spring Harb. Symp. Quant. Biol., *59:* 661–672, 1994.

3. Sutherland, R. M., Eddy, H. A., Bareham, B., Reich, K., and Vanantwerp, D. Resistance to Adriamycin in multicellular spheroids. Int. J. Radiat. Oncol. Biophys., *5:* 1225–1230, 1979.

4. Nederman. T., and Carlsson, J. Penetration and binding of vinblastine and 5-fluorouracil in cellular spheroids. Cancer Chemother. Pharmacol., *13:* 131–135, 1984.

5. Chaplin, D. J., Durand, R. E., and Olive, P. L. Cell selection from a murine tumour using the fluorescent probe Hoechst 33342. Br. J. Cancer, *51:* 569–572, 1985.

6. Durand, R. E. Distribution and activity of antineoplastic drugs in a tumor model. J. Natl. Cancer Inst., *81:* 146–152, 1989.

7. Teicher, B. A., Holden, S. A., Al-Achi, A., and Herman, T. S. Classification of antineoplastic treatments by their differential toxicity towards putative oxygenated and hypoxic tumor subpopulations *in vivo* in the FsaIIC murine fibrosarcoma. Cancer Res., *50:* 3339–3344, 1990.

8. Jain, R. K. Vascular and interstitial barriers to delivery of therapeutic agents in tumors. Cancer Metastasis Rev., *9:* 253–266, 1990.

9. Jain, R. K. Delivery of molecular and cellular medicine to solid tumors. Microcirculation, *1:* 1–23, 1997.

10. Tunggal, J. K., Cowan, D. S. M., Shaikh, H., and Tannock, I. F. Penetration of anticancer drugs through solid tissue: a factor that limits the effectiveness of chemotherapy for solid tumors. Clin. Cancer Res., *5:* 1583–1586, 1999.

11. Lankelma, J., Dekker, H., Luque, R. F., Luykx, S., Hoekman, K., van der Valk, P., van Diest, P. J., and Pinedo, H. M. Doxorubicin gradients in human breast cancer. Clin. Cancer Res., *5:* 1703–1707, 1999.

12. Tunggal, J. K., Melo, T., Ballinger, J. R., and Tannock, I. F. The influence of P-glycoprotein on the penetration of anticancer drugs through multicellular layers. Int. J. Cancer, *86:* 101–107, 2000.

13. Cowan, D. S. M., and Tannock, I. F. Factors that influence the penetration of methotrexate through solid tissue. Int. J. Cancer, *91:* 120–125, 2001.

14. Hicks, K. O., Ohms, S. J., van Zijl, P. L., Denny, W. A., Hunter, P. J., and Wilson, W. R. An experimental and mathematical model for the extravascular transport of a DNA intercalator in tumours. Br. J. Cancer, *76:* 894–903, 1997.

15. Wilson, W. R., and Hicks, K. O. Measurement of extravascular drug diffusion in multicellular layers. Br. J. Cancer, *79:* 1623–1626, 1999.

16. Vaupel, P., Kallinowski, F., and Okunieff, P. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. Cancer Res., *49:* 6449–6465, 1989.

17. Helmlinger, G., Yuan, F., Dellian, M., and Jain, R. K. Interstitial pH and pO_2 gradients in solid tumors *in vivo:* simultaneous high resolution measurements reveal a lack of correlation. Nat. Med., *3:* 177–182, 1997.

18. Cowan, D. S. M., Hicks, K. O., and Wilson, W. R. Multicellular membranes as an *in vitro* model for extravascular diffusion in tumours. Br. J. Cancer, *74* (Suppl. 27)*:* 528s–531s, 1996.

19. Phillips, R. M., Loadman, P. M., and Cronin, B. P. Evaluation of a novel *in vitro* assay for assessing drug penetration into avascular regions of tumours. Br. J. Cancer, *77:* 2112–2119, 1998.

20. Minchinton, A. I., Wendt, K. R., Clow, K. A., and Fryer, K. H. Multilayers of cells growing on a permeable support. An *in vitro* tumour model. Acta Oncol., *36:* 13–16, 1997.

21. Erkmen, K., Egorin, M. J., Reyno, L. M., Morgan, R., Jr., and Doroshow, J. H. Effects of storage on the binding of carboplatin to plasma proteins. Cancer Chemother. Pharmacol., *35:* 254–256, 1995.

22. Lindberg, P., Nordberg, P., Alminge, T., Mayer, L. D., Bally, M. B., and Cullis, P. R. Uptake of Adriamycin into large unilamellar vesicles in response to a pH gradient. Biochim. Biophys. Acta, *857:* 123–126, 1986.

23. Schindler, M., Grabski, S., Hoff, E., and Simon, S. M. Defective pH regulation of acidic compartments in human breast cancer cells (MCF-7) is normalized in Adriamycin resistant cells (MCF-7adr). Biochemistry, *35:* 2811–2817, 1996.

24. Durand, R. E. Slow penetration of anthracyclines into spheroids and tumors: a therapeutic advantage? Cancer Chemother. Pharmacol., *26:* 198–204, 1990.

25. Davis, A. J., and Tannock, I. F. Repopulation of tumour cells between cycles of chemotherapy: a neglected factor. Lancet Oncol., *1:* 86–93, 2000.

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