Angiogenesis in the Hollow Fiber Tumor Model Influences Drug Delivery to Tumor Cells: Implications for Anticancer Drug Screening Programs

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Abstract

The National Cancer Institute uses the hollow fiber assay as part of its screening program for anticancer drug discovery. Angiogenesis to hollow fibers implanted s.c. has not been reported, thereby raising concerns about the efficiency of drug delivery and its subsequent effects on chemosensitivity. By extending postimplantation times beyond the 6-day period presently used, extensive vascular networks develop, resulting in both increased delivery and chemosensitivity to doxorubicin. This study suggests that present protocols used to evaluate compounds may produce false negative results, and additional studies to determine the predictive value of the assay are required.

Introduction

In the late 1980s, the Developmental Therapeutics Program of the NCI radically changed its approach to the large-scale, random screening of compounds for anticancer activity (1). Instead of using xenografts or syngeneic rodent tumor models as a front line screen, all compounds submitted to the NCI are evaluated against a panel of well-characterized human tumor cell lines. Compounds are selected for further evaluation if they possess disease-specific activity in vitro, are COMPARE negative (i.e., have chemosensitivity fingerprints that differ from standard agents), or if activity in vitro is correlated with the expression of specific molecular targets in the cell line panel (1-4). It was envisaged that the primary in vitro screen would have such discriminatory powers that only a few agents would need to be evaluated against human tumor xenografts. This, however, has not been the case (5), thereby creating a backlog of compounds awaiting evaluation against xenografts. Because of the cost associated with the use of xenografts to evaluate large numbers of compounds, the NCI developed and introduced an intermediate screen through which compounds must pass before full evaluation in xenograft tumor models (6). The hollow fiber assay (7, 8) used by the NCI involves the short-term in vitro culture (1 day) of tumor cells inside biocompatible, polyvinylidene fluoride fibers, followed by implantation into mice at two anatomically separate sites (s.c. and i.p.). Drugs are administered i.p. (mice are treated daily for up to 4 days), fibers are removed 2 days later (total time in vivo is 6 days), and chemosensitivity is assessed using a modified MTT assay in vitro (8). Compounds are submitted for more extensive in vivo evaluation in xenografts if they demonstrate activity at both the s.c. and i.p. sites. Responses at the i.p. site might be expected to reflect in vivo chemosensitivity (although they are unlikely to be identical as drug exposure parameters will be different due to drug clearance from the peritoneal cavity), as it is unlikely that drug delivery problems would occur after an i.p. drug administration. Responses at the s.c. site after i.p. drug administration, however, may indicate that the new agent possesses both good bioavailability and pharmacological properties. Drug delivery to tumors is dependent on several factors, one of which is the presence of an established blood supply. Blood vessel development to hollow fibers implanted s.c. has not been reported previously, raising concerns about the efficiency of drug delivery and the subsequent effects this may have on chemosensitivity, bearing in mind the role that drug C × T values play in determining chemosensitivity (9). Lack of activity at the s.c. site may, therefore, be caused by poor drug delivery as a result of lack of blood vessel development; if this is the case, the validity of the test system is called into question. Tumor cells in hollow fibers should theoretically be able to induce an angiogenic response as the molecular weight cutoff point of the fiber walls is large enough (M, 500,000) to allow for appropriate growth factors to pass through the fiber wall. Present protocols for using the hollow fiber assay involve both short-term in vitro (1 day) and in vivo (6 day) cultivation, which may be too short to enable angiogenesis to occur. The aims of this study, therefore, were: (a) to determine whether hollow fibers implanted s.c. can develop a blood supply if postimplantation times are extended beyond the 6-day period presently used; and (b) to determine what effect the presence or absence of a vascular network has on both drug delivery and chemosensitivity in a murine colon tumor model.

Materials and Methods

Materials. Poly-Vinyl-Idene-Fluoride Spectra/Por Hollow fibers (internal diameter = 1 mm with a molecular weight cutoff point of M, 500,000) were purchased from Spectrum Medical, Inc. (Houston, TX). Doxorubicin, MTT, and DMSO were purchased from Sigma (Poole, Dorset, United Kingdom). Solvents for high-performance liquid chromatography analysis of doxorubicin were of analytical grade and were purchased from Fisher Scientific (Loughborough, United Kingdom). All cell culture material was purchased from Life Technologies, Inc. (Paisley, United Kingdom).

Animals. Pure strain NMRI mice (6-8 weeks of age) were obtained from B & K Universal, Ltd. (Hull, United Kingdom) and kept under 12 hourly cycles of light and darkness with access to a CRM pellet diet (Special Diet Services, Witham, United Kingdom) and water ad libitum. All animal experiments were conducted under appropriate animal licenses issued by the Home Office (London, United Kingdom), and United Kingdom Coordinating Committee on Cancer Research guidelines were followed throughout (10).

Cell Culture. MAC 15A cells were obtained from an ascitic murine adenocarcinoma of the colon, as described elsewhere (9), and routinely maintained as monolayer cultures in RPMI 1640 supplemented with FCS (10%), sodium pyruvate (2 mM), Penicillin/Streptomycin (50 IU ml 1/50 µg ml 1), and l-glutamine (2 mM) and buffered with HEPES (25 mM). Fibers were rehydrated, sterilized, and loaded with MAC 15A cells (1 × 106 cells ml 1), as described by Hollingshead et al. (8). Fibers were transferred to a T-25 flask containing growth medium and incubated at 37°C for a period of 14 days under constant agitation (80 rpm) on an orbital shaker, with daily changes of medium. Histological analysis of fibers after 14 days of culture in vitro demonstrated that fibers contain a central necrotic core (data not shown).
Fig. 1. Morphological appearance of blood vessel development to hollow fibers implanted s.c. into NMRI mice. A, typical appearance of subdermal vasculature in the absence of fibers. Blood vessel development to fibers containing MAC 15A cells on days 4 (B), 7 (C), 14 (D), 21 (E), 28 (F), and 32 (G) after implantation at the s.c. site. H, absence of blood vessel development to fibers containing medium only (28 days after implantation s.c.).
characterized cells (including patterns of growth factor expression) can be implanted into fibers, and that these cells can be retrieved intact at various stages after implantation without host cell infiltration. Additional studies are required, however, to determine whether angiogenesis in the hollow fiber model has advantages over presently existing techniques (13), particularly with respect to quantitation, the use of windows to observe blood vessel development, and the use of fibers containing gels impregnated with growth factors.

The presence or absence of a vascular supply to hollow fibers implanted s.c. has a significant effect on both drug delivery (Table 1) and chemosensitivity (Table 2). In terms of drug delivery, the concentration of doxorubicin in hollow fibers (s.c.) containing MAC 15A cells (Table 1) is significantly higher when doxorubicin is administered 28 days after implantation (0.43 ± 0.35 ng mg⁻¹) compared with the same fibers treated 4 days after implantation (<0.015 ng mg⁻¹). In hollow fibers (s.c.) containing medium only, no significant differences exist in doxorubicin levels inside fibers treated either 28 or 4 days after implantation (Table 1), which reflects the fact that development of a blood vessel supply does not occur in blank fibers (Fig. 1). Similarly in fibers implanted i.p. post implantation, times have no significant effect on drug concentrations inside the fiber, although much greater concentrations are achieved compared with fibers implanted s.c. (Table 1). The relationship between drug distribution and blood vessel development is also reflected in terms of chemosensitivity in that s.c. fibers treated when a vascular supply is established (day 28) are more sensitive to doxorubicin than s.c. fibers treated in the absence of a vascular supply (Table 2). No significant difference exists between the response of MAC 15A cells in fibers implanted i.p. for 4 or 28 days before drug administration (Table 2).

The results of this study demonstrate that the treatment of hollow fibers s.c. with doxorubicin in the absence of an established blood supply gives rise to a false negative result largely to the fact that drug delivery is impaired. In terms of drug screening, experimental models must meet several criteria, one of which being that the predictive value of the model is such that false negative results are kept to a minimum. In the case of the hollow fiber assay, no published information is available on the predictive value of the hollow fiber model in terms of whether or not responses in hollow fibers correlate with activity in xenografts. Hollingshead et al. (8) have demonstrated activity, however, against fibers implanted s.c. for several standard anticancer agents, although it is not known how drugs reach this site in the absence of a vascular supply. As impaired drug delivery to tumors is a major obstacle for an effective anticancer drug therapy, it could be argued that compounds that are active against hollow fibers at the s.c. site have the highly desirable property of being efficiently delivered to tumors in the absence of a good blood supply. In addition, modifications to the hollow fiber assay to commence chemotherapy once a blood supply has been established would lead to more compounds going through to testing against xenograft models, resulting in possible logistical problems for large-scale screening programs. Nevertheless, the results of this study raise the possibility that potentially more compounds against xenograft models must meet several criteria, one of which being that the predictive value of the hollow fiber model is such that false negative results are kept to a minimum. In the case of the hollow fiber assay, no published information is available on the predictive value of the hollow fiber model in terms of whether or not responses in hollow fibers correlate with activity in xenografts. Hollingshead et al. (8) have demonstrated activity, however, against fibers implanted s.c. for several standard anticancer agents, although it is not known how drugs reach this site in the absence of a vascular supply. As impaired drug delivery to tumors is a major obstacle for an effective anticancer drug therapy, it could be argued that compounds that are active against hollow fibers at the s.c. site have the highly desirable property of being efficiently delivered to tumors in the absence of a good blood supply. In addition, modifications to the hollow fiber assay to commence chemotherapy once a blood supply has been established would lead to more compounds going through to testing against xenograft models, resulting in possible logistical problems for large-scale screening programs. Nevertheless, the results of this study raise the possibility that potentially more compounds against xenograft models are required.
In conclusion, this study has demonstrated that hollow fibers containing tumor cells can induce angiogenesis in mice and this has direct implications for drug delivery and chemosensitivity. These results suggest that the treatment of fibers before blood vessel development occurs may be a source of false negative predictions, and studies to address this question need to be conducted. Finally, the demonstration that hollow fiber tumor models can induce an angiogenic response in the host suggests that this model may have some use in the search for antiangiogenic drugs, although considerable refinement of the assay to address questions such as quantitation of blood vessel development need to be addressed.

References

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