Pharmacokinetics of PK1 and Doxorubicin in Experimental Colon Tumor Models with Differing Responses to PK1

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ABSTRACT

PK1 is a synthetic N-(2-hydroxypropyl)methacrylamide copolymer-doxorubicin (dox) conjugate currently undergoing Phase II evaluation in the United Kingdom. We have studied the activity of PK1 in three murine colon tumor models that differ in terms of morphology and vascularization in an attempt to determine which factors are most important in the tumor response to PK1. Vascular permeability was evaluated with Evans Blue, and pharmacokinetic studies in MAC15A and MAC26 used high-performance liquid chromatography to monitor both PK1 uptake and dox release in the tumors. Cathepsin B activity was assessed using a specific substrate. PK1 (40 mg/kg1 dox equivalent) was significantly more effective than dox alone (10 mg/kg−1) was against MAC15A tumors, which possess enhanced perfusion and retention, but not against MAC26 tumors, although MAC15A was also responsive to PK1 when grown as avascular micrometastatic deposits in the lung. Pharmacokinetic studies showed similar levels of PK1 in both tumors. Peak tumor levels of released dox were 7-fold greater in the responsive MAC15A tumor (53 μg/ml−1) compared with the less responsive MAC26 tumor (7.7 μg/ml−1) and more than 18-fold greater in MAC15A than when free dox was given. These differences in response correlated also with an increased lysosomal activity of cathepsin B. Calculated AUCs for intratumoral dox released were 431 μg.h/g−1 and 775 μg.h/g−1 for MAC15A and MAC26, respectively. These AUCs are 4-fold and 7-fold higher, respectively, than when dox is given alone. This study has shown that activity and the pharmacokinetics of PK1 and released dox are dependent on both the vascular properties and enzyme content of the tumors. These studies are likely to have clinical implications as aggressive tumors are known to have increased protease activity.

INTRODUCTION

dox3 is an anthracycline antibiotic with activity against a broad spectrum of tumors (1). The therapeutic index of dox is, however, compromised by two factors common to most anticancer agents, toxicity and resistance. Several different formulations of dox have been investigated to improve its delivery to tumor and to decrease the dose-limiting cardiotoxicity (2) associated with the drug. Acquired cellular resistance to dox via overexpression of P-glycoprotein encoded by the mdr1 gene is well-documented (3, 4).

Drug-polymer conjugates are now being developed to try to enhance the selectivity of some of the more established anticancer agents (5, 6). PK1 is a synthetic HPMA copolymer-dox conjugate, the dox being linked to the polymer by a Gly-Phe-Leu-Gly peptidyl spacer (Fig. 1). PK1 has a molecular weight of approximately M, 25,000 and is essentially nontoxic in vitro (5). This high molecular weight restricts uptake of the conjugate into the tumor cell to the process of endocytosis. This results in lysosomal concentration of the polymer conjugate in which the peptidyl linker is degraded by the thiol- dependent lysosomal proteases releasing free dox within the cell (7). The molecular weight of the polymer is also critical in determining the clearance of the polymer itself from both the tumor and the systemic circulation (8). This method of delivering dox to tumors has been shown to reduce cardiotoxicity in rats (9) and recently to overcome the problem of mdr1 gene-encoded multidrug resistance (10). PK1 has shown promising preclinical antitumor activity against several solid tumors (11), has now progressed through Phase I clinical trials (12), and is undergoing Phase II evaluation in the United Kingdom.

The pharmacokinetics of PK1 have been studied previously in non-tumor-bearing mice (13), and, in studies with tumor-bearing mice, the polymer has been shown to increase the levels of dox in B16F10 melanoma (14). PK1 is thought to accumulate in tumor tissue as a result of EPR. The EPR effect relies on the fact that tumor vasculature is uniquely different from that of normal tissue in that it is permeable to macromolecules and plasma proteins (15).

Variation in the response of solid tumors to copolymer conjugates has been shown previously (11). It is thought these differences may be explained by the extent of vascular permeability, which is important for passive tumor targeting and tumor distribution of the conjugate, and appropriate lysosomal enzyme

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3 The abbreviations used are: dox, doxorubicin; HPMA, N(2-hydroxypropyl)methacrylamide; EPR, enhanced permeability and retention; MAC, murine adenocarcinoma of the colon; HPLC, high-performance liquid chromatography; MTD, maximum tolerated dose; AUC, area under the time/concentration curve.
levels. Steyger et al. (16) also suggested that intratumoral distribution of the polymer may be important in explaining these differences because it is acknowledged that EPR may play an important role in tumor response. We, therefore, investigated the activity of PK1 in MAC models, which differ in terms of morphology and vascularization (17).

The MAC15A and MAC26 tumors selected for therapeutic studies are resistant experimental colon tumors that have been used widely in the preclinical evaluation of potential anticancer agents (18–20). The differences in morphology and vascularization suggest they are likely to differ in their vascular permeability and hence in their abilities to take up PK1. MAC15A is a poorly differentiated, rapidly growing adenocarcinoma that becomes necrotic, and tumor cells are associated with blood vessels in typical tumor cords (17). On the other hand, MAC26 is a well-differentiated glandular adenocarcinoma with a clear stromal component and well-developed blood supply. There is a considerable amount of information in the literature suggesting that tumors differ greatly in their expression of proteases, in particular cysteine proteases such as cathepsin B (21), and differences in cathepsin B activity may influence the release of dox. In addition to studies on the activity of PK1 against s.c. transplanted MAC15A and MAC26, MAC 15A tumor grown as avascular micrometastatic deposits in the lung was also investigated. Not only is this a clinically relevant model, but it should provide a useful comparison to the other solid tumors because EPR cannot play a role in the response of these deposits. The response of such models should give further important information on the factors that influence the antitumor activity of PK1.

To correlate EPR status of these tumors with the tumor response to PK1, the vascular permeability of these tumors was first evaluated with Evans Blue (22). Evans Blue is a dye that complexes with plasma albumin and, thus, can be used as a

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Fig. 1 Structural formula.

Fig. 2 Antitumor effects of dox or PK1 against MAC26 tumors (a), MAC15A (b,c). Points, mean relative tumor volumes (n > 5); ○, PK1 (40 mg·kg⁻¹); □, PK1 (20 mg·kg⁻¹); △, PK1 (10 mg·kg⁻¹); ▽, dox (10 mg·kg⁻¹); ◊, controls.
Pharmacokinetics of PK1

Cancer Research guidelines (23) were followed throughout. Kingdom, and United Kingdom Co-Ordinating Committee on project license approved by the Home Office, London, United Kingdom, and all of the chemicals were purchased from Sigma grade purchased from Fisher Scientific (Loughborough, United Kingdom). All of the solvents were of HPLC.

Fig. 3 Relative perfusion of MAC15A (○) and MAC26 (△) tumors measured by Evans Blue concentration.

marker of albumin extravasation. Evidence of an increased EPR effect should indicate increased uptake of PK1 into tumors, whereas evidence of increased thiol-dependent protease activity should indicate increased dox release within the tumor. Therefore, cathepsin B activity was measured using a specific peptide substrate. Pharmacokinetic studies were used to monitor both the rate of uptake of PK1 into tumors and the rate of intratumoral dox released from PK1. This information may help to explain differences seen and give a clearer picture of the role EPR and thiol-dependent enzyme activity plays in the response of such tumors to macromolecular prodrugs.

The aims of this study were, therefore, threefold: (a) to study activity of PK1 compared with dox against two transplantable syngeneic tumors with differing morphological characteristics; (b) to investigate activity against clinically relevant avascular lung micrometastases of MAC15A; and (c) to determine the plasma pharmacokinetics and tumor uptake of the polymer PK1 and released dox in tumor models with different EPR and enzymatic properties. The information obtained will allow determination of whether the pharmacokinetics of PK1 or released dox correlates with tumor response.

MATERIALS AND METHODS

Chemicals and Reagents

PK1 was kindly supplied by Polymer Laboratories (Church Stretton, United Kingdom). All of the solvents were of HPLC grade purchased from Fisher Scientific (Loughborough, United Kingdom), and all of the chemicals were purchased from Sigma (Poole, United Kingdom).

Antitumor Activity

All of the animal experiments were carried out under a project license approved by the Home Office, London, United Kingdom, and United Kingdom Co-Ordinating Committee on Cancer Research guidelines (23) were followed throughout.

Table 1 Comparative activity of dox (10 mg · kg⁻¹) and PK1 (40 mg · kg⁻¹ dox equivalent) against a model of avascular colon carcinoma micrometastases (MAC15A)

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of colonies</th>
<th>Mean ± 1 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>139, 141, 175, 219ᵃ</td>
<td>168 ± 38</td>
</tr>
<tr>
<td>PK1</td>
<td>0, 2, 5, 6, 7, 14</td>
<td>5.7 ± 4.8ᵇ</td>
</tr>
<tr>
<td>dox</td>
<td>0, 0, 1, 3, 8, 11, 58</td>
<td>11.5 ± 21ᵇ</td>
</tr>
</tbody>
</table>

ᵃ Denotes 2 of 6 tumor-associated deaths.
b P = 0.01.

s.c. Tumors. Two tumors (MAC15A and MAC26) with different morphologies and vascularization were grown s.c. in Naval Medical Research Institute pure-strain mice (B&K Universal, Hull, United Kingdom). MAC15A is an ascitic variant of the solid MAC15 but in this study was grown as a solid tumor when ascites cells (1 × 10⁶) were injected s.c. MAC26 was transplanted as single tumor fragments in the flank by trocar. Groups of 5–10 tumor-bearing mice were treated with dox at the previously established single i.v. MTD of 10 mg·kg⁻¹ or PK1 at equivalent dox concentrations of 10–40 mg·kg⁻¹ [40 mg·kg⁻¹ (dox equivalent) is the MTD of PK1]. Treatment commenced when tumors could be reliably measured by calipers (mean dimensions, 7 × 10 mm), and therapeutic effects were assessed by twice weekly caliper measurements of the tumor. Tumor volumes were determined by the formula a² × b/2 where a is the smaller and b is the larger diameter of the tumor. Graphs were plotted of relative tumor volume against time and anti tumor activities assessed by Mann-Whitney analysis.

Lung Nodules. Lung nodules were established after i.v. inoculation of MAC 15A cells. Groups of 6–7 NMRI female mice were injected with approximately 1 × 10⁶ MAC15A cells via the tail vein. Treatment was either dox (10 mg·kg⁻¹) or PK1 (40 mg·kg⁻¹ dox equivalents) by single tail vein injection and comparisons were made with control animals when the first signs of tumor-associated stress occurred. Lungs were removed from sacrificed animals and fixed in Bouin’s fluid, and tumor surface colonies counted. Differences between treated and control groups were assessed using Student’s t test.

Blood Vessel Leakage

Tumor blood vessel leakage was determined by an Evans Blue dye-perfusion technique (22). Evans Blue dye (10 mg·ml⁻¹, 250 μl) was injected i.v. into the tail vein of 25-g NMRI mice bearing s.c. tumors. Tumors of uniform size, equivalent to those used for therapeutic studies, were selected for perfusion studies. Mice were killed and tumors were removed 2 min and 6, 24, and 48 h after injection, and the dye was extracted from tumors and measured spectrophotometrically (22). Relative perfusion of Evans Blue was compared in the two tumor models using the paired Student’s t test.

Cathepsin B Activity

Tumors were prepared for assay by homogenizing in PBS (pH 7.4) at 4°C using a glass Potter homogenizer. Homogenates were centrifuged at 2000 × g for 10 min to isolate the nuclear fraction, and the supernatant was further centrifuged at 8000 × g for 20 min to isolate the lysosomal fraction. The pellet from this fraction was washed twice in PBS before being suspended
in citrate phosphate buffer [0.2 M (pH 5.5)] containing freshly prepared reduced glutathione (5 mM), EDTA (1 mM), and Triton X-100 (0.2%), and samples were sonicated to release lysosomal enzymes. All of the samples were kept at 4°C throughout. Protein concentrations were measured using the Bradford assay (24). Cathepsin B activity was measured using the method of Turk and Kregar (25) with the synthetic substrate n-CBZ-Phe-Arg-4-methoxy-naphthylamide (Sigma). Activity was calculated by determining the amount of naphthylamide released from the substrate per unit time.

Fig. 4 A, dox concentrations (mean ± 1 SD) in MAC15A (○) and MAC26 (△) plasma (dotted line) and tumor (solid line) after administration of 10 mg·kg⁻¹ dox i.v. (n = 3). B, PK1 concentrations (mean ± 1 SD) in MAC15A (○) and MAC26 (△) plasma (dotted line) and tumor (solid line) after administration of 490 mg·kg⁻¹ PK1 i.v., [40 mg·kg⁻¹ dox equivalent (n = 3)]. C, free dox concentrations (mean ± 1 SD) in MAC15A (○) and MAC26 (△) plasma (dotted line) and tumor (solid line) after administration of 490 mg·kg⁻¹ PK1, i.v. [40 mg·kg⁻¹ dox equivalent (n = 3)].

Table 2  Pharmacokinetic parameters for dox after administration of dox 10 mg·kg⁻¹ i.v. to tumor-bearing NMRI mice

<table>
<thead>
<tr>
<th></th>
<th>T_max</th>
<th>C_max</th>
<th>t½</th>
<th>AUC_C_last</th>
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<tbody>
<tr>
<td></td>
<td>(h)</td>
<td>(µg· ml⁻¹)</td>
<td>(h)</td>
<td>(µg· h· ml⁻¹)</td>
</tr>
<tr>
<td>MAC15A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>0.083</td>
<td>4.2</td>
<td>15</td>
<td>5.6</td>
</tr>
<tr>
<td>Tumor</td>
<td>4</td>
<td>2.9</td>
<td>27.6</td>
<td>105</td>
</tr>
<tr>
<td>MAC26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>0.083</td>
<td>16</td>
<td>17</td>
<td>7.8</td>
</tr>
<tr>
<td>Tumor</td>
<td>4</td>
<td>2.6</td>
<td>28.3</td>
<td>100</td>
</tr>
</tbody>
</table>

a T_max, corresponding time; C_max, maximum concentration.
Pharmacokinetic Studies

Tumors. The two tumors used for pharmacokinetic studies (MAC15A and MAC26) were grown s.c. in male NMRI pure-strain mice as described above. Groups of tumor-bearing mice were treated with dox at the previously established single i.v. MTD of 10 mg kg\(^{-1}\) or PK1 at the previously established single i.v. MTD of 490 mg kg\(^{-1}\) (equivalent dox concentration of 40 mg kg\(^{-1}\)). Treatment commenced when tumors could be reliably measured by calipers.

Pharmacokinetics. At various time points after the administration of dox or PK1, mice were anesthetized, and blood samples were taken via cardiac puncture. The blood was kept at 4°C until centrifugation (1000 g for 5 min at 4°C), and the plasma was separated and stored at −20°C. Tumors were taken and placed immediately into liquid nitrogen with three mice being used per time point. The pharmacokinetic parameters were estimated by standard noncompartmental methods. The terminal elimination rate (\(K_{el}\)) was calculated using linear regression analysis of the terminal log linear portion of the curve. The area under the plasma concentration versus time curve (AUC) was calculated using the trapezoidal rule.

**dox Extraction.** dox was extracted from plasma and tumors using a method based on that of Fraier et al. (26) after homogenization of the tumors in PBS. A 50-μl aliquot of the sample was spiked with 10 μl of daunorubicin (4 μg ml\(^{-1}\)) and extracted into 250 μl of isopropanol-chloroform (25:75, v/v) by vortex mixing for 1 min. After centrifugation, the upper aqueous phase was separated and mixed with 80 μl of borate buffer [0.05 (pH 8.4)] and re-extracted as described above. The two organic phases were combined and evaporated to dryness. The residue was redissolved in 200 μl of methanol 0.5 M H\(_2\)PO\(_4\) (50:50, v/v), and the solution was washed with 600 μl of hexane. After washing, the hexane was discarded, and the aqueous layer was injected into the HPLC system.

**PK1 Extraction.** To permit the accurate quantification of polymer-bound dox, it was necessary to release the free drug from the conjugate by acid hydrolysis. The acid hydrolysis would release a highly fluorescent aglycone (doxorubicinone) that could be quantified by HPLC (26). For acid hydrolysis, 50 μl of sample was exposed to 50 μl of 1 M HCl at 85°C for 20 min. After cooling to room temperature, 10 μl of daunorubicin (4 μg ml\(^{-1}\)), 50 μl of 0.5 μl phosphate buffer (pH 7.4), and 50 μl of 1 M sodium hydroxide were added. Subsequent extraction with isopropanol-chloroform was the same as for the free drug but the extraction step with borate buffer was omitted.

**Chromatography.** Samples were then analyzed by a standard HPLC method using fluorescence detection (27). This method was capable of separating several dox metabolites including the aglycone; therefore, bound dox (PK1) was estimated by the subtraction of the free aglycone and dox from the total aglycone after acid hydrolysis. Calibration curves for both dox and PK1 using drug-free mouse plasma were linear over the range 0.01–1 μg ml\(^{-1}\).

**In Vitro Drug Release Studies.** Tumor lysosomal fractions at a concentration of 2 mg ml\(^{-1}\) protein in citrate buffer (as described for cathepsin B) were spiked with PK1 at the dox equivalent of 80 μg ml\(^{-1}\). At hourly intervals, 30 μl of sample was removed and assayed for free dox by HPLC.

**RESULTS**

Effects of dox or PK1 on s.c. tumor growth are presented in Fig. 2a–c. dox alone was not very effective against either of the tumors with only a modest growth delay achieved at the MTD. This dose resulted in a maximum body weight loss of 4%. PK1 was not significantly more effective than dox alone against the MAC26 tumor (Fig. 2a). Against MAC15A (Fig. 2b), PK1 at 10 mg kg\(^{-1}\) was only as effective as dox, but increasing the dose level to 40 mg kg\(^{-1}\) dox equivalent (Fig. 2c) resulted in highly significant antitumor activity (\(P < 0.01\)). This dose level was well tolerated, with body weight loss being only 5.4%.

Results from tumor blood perfusion studies are presented in Fig. 3. Data indicate increased concentration (\(C_{max}\)) at hourly intervals, \(t_{max}\), and peak plasma levels of dox after administration at 10 mg kg\(^{-1}\) (Fig. 4a; Table 2) are similar to published values (28) with peak plasma levels of 4 and 16 μg ml\(^{-1}\) for MAC15A- and MAC26-bearing mice.
respectively. Both MAC15A- and MAC26- bearing mice have almost identical terminal half-lives for the dox in plasma and tumor giving similar AUCs in both sets of mice. Plasma AUCs were 5.6 and 7.8 μg·h·ml⁻¹ and tumor AUCs 105 and 100 μg·h·ml⁻¹ for MAC15A and MAC26, respectively.

This study has shown that the administration of dox as the HPMA copolymer conjugate results in quite different free dox pharmacokinetics when compared with administration of free dox. This is similar to previous reports (13). The plasma and tumor concentrations of the dox conjugate PK1 are shown graphically in Fig. 4b, and the pharmacokinetic parameters are given in Table 3. PK1 has similar peak plasma levels (approximately 2,500 μg·ml⁻¹) in both sets of mice as may be expected, although PK1 is cleared from the plasma more quickly in mice bearing MAC15A than in mice bearing MAC26, which results in AUCs of 13,000 and 35,000 μg·h·ml⁻¹, respectively, dox equivalents from these levels of PK1 are given in Table 3. Tumor levels seem to be similar, although the peak levels of 187 μg·ml⁻¹ occur in MAC15A after 1 h, whereas peak levels of 137 μg·ml⁻¹ in MAC26 tumors are not achieved until 6 h.

The concentrations of dox released after administration of PK1 are shown in Fig. 4c, and the parameters are given in Table 4. There is a 20-fold increase in peak tumor concentrations of released dox in the responsive MAC15A (53 μg g⁻¹) compared with dox alone (2.9 μg g⁻¹) but only a 3-fold increase in the less responsive MAC26 tumor (7.70 μg g⁻¹). These peak concentrations of released dox are seen earlier in the MAC15A tumor (2 h) than the MAC26 tumor (6 h). Elimination of released dox from MAC15A was faster (τ₁/₂ = 12.2 h) resulting in an AUC of 431 μg·h·g⁻¹ compared with 775 μg·h·g⁻¹ for MAC26 (τ₁/₂ = 66 h). These AUCs are 4-fold and 7-fold higher, respectively, than when dox is given alone. Plasma concentrations of released dox are consistently low in MAC15A-bearing mice without the characteristic high peak plasma seen after i.v. administration of dox alone, but the plasma half-life is long (>30 h), which gives an AUC greater than for dox alone.

In vitro studies showed that MAC15A lysosomal fractions have a higher cathepsin B activity than the equivalent preparation from MAC26. Calculated activity was 111 ± 11.0 μmol/min/mg for MAC15A compared with 71.8 ± 8.5 μmol/min/mg for MAC26. This increased cathepsin B activity was also reflected in the in vitro release profile of dox from PK1 when incubated in a similar system. dox was released from PK1 at a rate of 33 ± 1.32 nmol/min/mg for MAC15A and 11 ± 0.77 nmol/min/mg for MAC26.

DISCUSSION

This study has shown PK1 to be more active than dox alone against the generally refractory syngeneic murine colon tumor MAC15A when given as a single i.v. bolus. Activity of PK1 against a second tumor (MAC26) was no better than dox alone even at the MTD of 40 mg·kg⁻¹ dox equivalent. These data seem to correlate well with those from the Evans Blue perfusion experiment. MAC15A showed increased uptake of Evans Blue with time, which suggests that macromolecules can penetrate the vasculature of this tumor (and be retained within the tumor) more easily than that of MAC26. This difference in EPR would help to explain the differences in antitumor activity of PK1 seen between the tumors. The EPR effect however cannot explain the responses seen in the lung nodules to PK1. The micrometastases produced in this i.v. model are avascular (29) and yet were as equally sensitive to PK1 as to dox alone. The response of such an avascular model suggests that it is dox released from PK1 that is penetrating the avascular tumor mass.

The pharmacokinetic studies described here showed low dox concentrations detected in the plasma of mice bearing MAC15A(s.c.) tumors after the administration of PK1, and, although the plasma levels were low, they were persistent. These low persistent plasma levels of dox provide a likely explanation for the responses seen in the avascular micrometastatic deposits. The high peak plasma levels of dox seen when dox is given alone are absent after PK1 administration giving a possible explanation for the lack of cardiotoxicity as suggested previously (13) though other anthracycline associated toxicities caused by PK1 are seen in rodents (30). The low plasma levels of dox after PK1 administration may have a definite therapeutic advantage. Previous experimental studies have shown that split-dose scheduling of dox giving low, prolonged plasma levels can increase tumor response dramatically (31). Split-dose scheduling was aimed at decreasing systemic toxicity by decreasing peak plasma concentrations though these high peak concentrations are desirable in a tumor but difficult to achieve after systemic administration.

In the present study, tumor concentration profiles of the polymer conjugate show high levels of PK1 retained in both MAC15A and MAC26 tumors beyond 70 h. These peak tumor levels of polymer-bound dox are similar in both MAC26 and MAC15A and comparable with those seen previously in B16F10 melanoma (14), however concentrations of dox released are considerably higher in the responsive MAC15A tumors. MAC15A showed a greater than 18-fold increase in peak tumor concentration of released dox compared with dox administered alone, whereas the equivalent value for the less responsive MAC26 was less than 3-fold higher. Dox is also released more quickly in the responsive MAC15A with peak tumor levels seen after 2 h compared with 6 h for the less responsive MAC26. The more rapid uptake of PK1 into MAC15A may be expected after the results observed with Evans Blue showing increased permeability of the MAC15A vasculature.

Dox is released from PK1 after pinocytic capture of the conjugate and enzymatic degradation by lysosomal, thiol-dependent cathepsins (8). The increased cathepsin B activity in MAC15A together with the more rapid release of dox from PK1 in tumor preparations in vitro would provide a further explanation for the higher and faster appearing dox peak in MAC15A than MAC26 tumors.

The experiments described here have shown that PK1 is not only more active than dox in selected solid tumors, it is equally active against avascular micrometastatic deposits. The comparison of the pharmacology in the two tumor models with differing responses to PK1 has highlighted various factors that may influence the activity of PK1. The pharmacokinetics of PK1 and released dox are clearly different in the two tumor models. This reflects differences in the vascular properties of the two tumors and also in their content of thiol-proteases, particularly cathepsin B. The protease expression of aggressive clinical tumors is known to vary, but this is the first report in which activity of PK1 has been correlated with lysosomal cathepsin activity. The importance of the
vascular component is further complicated by the recent studies of Fukumura et al. (32). They suggest that both the vessel density and vascular permeability of tumors may differ in experimental models depending on which site they are grown because vascular permeability depends not only on the expression of VEGF but also on the origin of the vessels (32).

We, therefore, intend to continue these studies with PK1 by investigating its disposition in orthotopic models where tumors are grown in more clinically relevant sites. This should give valuable information allowing the administration of PK1 and other macromolecular prodrugs to be optimized in the clinic.

REFERENCES

15. Matsumura, Y., and Maeda, H. A new concept for macromolecular therapeutics in cancer therapy: mechanisms of tumorotropic accumula-
