Phase I Clinical and Pharmacokinetic Study of PK1 [*N*-(2-Hydroxypropyl)methacrylamide Copolymer Doxorubicin]: First Member of a New Class of Chemotherapeutic Agents—Drug-Polymer Conjugates[1]

Paul A. Vasey,[2] Stan B. Kaye, Rosemary Morrison, Chris Twelves, Peter Wilson, Ruth Duncan, Alison H. Thomson, Lilian S. Murray, Tom E. Hilditch, Tom Murray, Sally Burtles, D. Fraier, E. Frigerio, and Jim Cassidy, on behalf of the Cancer Research Campaign Phase I/II Committee

CRC Department of Medical Oncology, Beatson Oncology Centre, Glasgow, United Kingdom, G11 6NT [P. A. V., S. B. K., R. M., C. T., P. W.]; The School of Pharmacy, University of London, London, United Kingdom, WC1N 1AX [R. D.]; Departments of Medicine and Therapeutics [A. H. T., S. M.] and Clinical Physics and Bio-Engineering [T. E. H.] and Radionuclide Dispensary [T. M.], Western Infirmary, Glasgow, United Kingdom, G116NT; Cancer Research Campaign, London, United Kingdom [S. B.]; Bioanalytical Laboratory, Pharmacokinetics and Metabolism Department, Pharmacia and Upjohn, Nerviano, Italy [D. F., E. F.]; Department of Medicine and Therapeutics, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, United Kingdom, AB25 2ZD [J. C.]

ABSTRACT

PK1 comprises doxorubicin covalently bound to *N*-(2-hydroxypropyl)methacrylamide copolymer by a peptidyl linker. Following cellular uptake via pinocytosis, the linker is cleaved by lysosomal enzymes, allowing intratumoral drug release. Radically altered plasma and tumor pharmacokinetics, compared to free doxorubicin, and significant activity in animal tumors have been demonstrated preclinically. We aimed to determine the maximum tolerated dose, toxicity profile, and pharmacokinetics of PK1 as an i.v. infusion every 3 weeks to patients with refractory or resistant cancers.

Altogether, 100 cycles were administered (range, 20–320 mg/m^2 doxorubicin-equivalent) to 36 patients (20 males and 16 females) with a mean age of 58.3 years (age range, 34–72 years). The maximum tolerated dose was 320 mg/m^2, and the dose-limiting toxicities were febrile neutropenia and mucositis. No congestive cardiac failure was seen despite individual cumulative doses up to 1680 mg/m^2. Other anthracycline-like toxicities were attenuated. Pharmacokinetically, PK1 has a distribution $t_{1/2}$ of 1.8 h and an elimination $t_{1/2}$ averaging 93 h. ^{131}I-labeled PK1 imaging suggests PK1 is taken up by some tumors. Responses (two partial and two minor responses) were seen in four patients with NSCLC, colorectal cancer, and anthracycline-resistant breast cancer.

PK1 demonstrated antitumor activity in refractory cancers, no polymer-related toxicity, and proof of principle that polymer-drug conjugation decreases doxorubicin dose-limiting toxicities. The recommended Phase II dose is 280 mg/m^2 every 3 weeks. Studies are planned in colorectal, NSCLC, and breast cancer patients.

INTRODUCTION

The anthracycline antitumor antibiotics are among the most useful agents in the treatment of solid malignancies, having a wide range of antitumor activity. Doxorubicin hydrochloride (Adriamycin) is the most commonly used and is effective in the treatment of carcinomas of the breast, lung, thyroid, ovary, and soft tissue sarcomas. However, anthracycline therapy is associated with significant general organ toxicities, especially myelosuppression, mucositis, and cardiac toxicity. Anthracyclines (like most cytotoxic agents) are low molecular weight compounds and, following i.v. administration, distribute readily into almost all tissues and intracellular compartments by rapid passage across the plasma membrane via passive diffusion or active transport (1). The resulting high peak concentration is an important factor in the development of toxicity (2), although the relevance of this pharmacokinetic effect to antitumor efficacy is less clear because there is minimal selective tumor tissue concentration (3). Novel forms of drug delivery designed to improve distribution by controlled release mechanisms could theoretically prolong the exposure of tumors to effective drug concentrations while decreasing peak plasma concentrations (4). However, prolonged infusions of anthracyclines produce different dose-limiting effects, such as dermatological toxicity (5).

Macromolecular systems have been described in which drug is either complexed with a carrier, *e.g.*, anthracycline-DNA (6) or cisplatin-polypeptide (7), or covalently bound to a carrier, *e.g.*, anthracycline-dextran (8) or anthracycline-proteins (9). Such carriers have been shown to alter drug pharmacokinetics at the whole organism and cellular level and facilitate controlled drug release at the tumor site. It has been shown that macromolecules such as albumin and polymer-drug/protein conjugates passively accumulate within solid tumor tissue (10). This phe-

Received 6/23/98; revised 8/27/98; accepted 8/28/98.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by the Cancer Research Campaign (London).
[2] To whom requests for reprints should be addressed, at CRC Department of Medical Oncology, Beatson Oncology Centre, Glasgow, United Kingdom, G11 6NT. Phone: 44 141 211 2627; E-mail: pav1y@clinmed.gla.ac.uk.

nomenon has been termed the EPR[3] effect and is thought to be due to tumor vasculature displaying a discontinuous endothelium, which allows macromolecular extravasation into tumor tissues, and also the lack of effective lymphatic drainage in tumors, which prevents efficient CL of such accumulated macromolecules (11, 12).

Drug conjugation to a macromolecular carrier has the principal effect of limiting cellular uptake to the mechanism of pinocytosis (13). Here, cellular internalization of the macromolecule occurs via membrane invagination, with immediate transfer to the endosomal compartment of the cell. A series of vesicle fusion events follow, with the macromolecule eventually being transferred to the lysosomal compartment and subsequently exposed to lysosomal enzymes (14). This restriction of drug uptake to the lysosomotropic route allows the exploitation of the opportunities for both passive and active targeting of tumors (15). In addition, such specific intracellular delivery of anthracyclines could provide a means of at least partially overcoming the P-glycoprotein cell surface membrane pump, responsible for the MDR phenotype (16).

The proposal that water-soluble polymers could function as carriers of drugs and that conjugation with a biodegradable spacer/linker could facilitate targeted drug release was first put forward in the mid-1970s (17). An optimal polymeric drug carrier system would ideally have: (a) a nonimmunogenic polymer, readily excreted or biodegraded by the host; (b) a linker that is stable in the circulation and degrades at a controlled rate at the site of action; and (c) a stable formulation that is amenable to commercial production [reviewed by Duncan (18)]. HPMA homopolymer is closely related to the soft contact lens material HEMA and was originally developed in Czechoslovakia as a plasma expander (19). It is hydrophilic and known to be nontoxic in the rat, even at doses of 30 g/kg. HPMA homopolymers and copolymers are chemically inert and are nonimmunogenic when covalently bound to anthracyclines (20, 21). HPMA copolymer doxorubicin (PK1, FCE28068), shown in Fig. 1, has a peptidyl linker (Gly-Phe-Leu-Gly) that is stable in the plasma (22) and has been shown to concentrate within solid tumor models (23, 24). It is then cleaved intracellularly by lysosomal cysteine proteinases (25), thereby allowing intratumoral drug release (Fig. 2). It has a molecular weight of ~30,000 (doxorubicin content, ~8% weight/2% mol) and is, therefore, too large to cross the cell membrane by diffusion. Preclinical work has shown that PK1 demonstrates radically different pharmacokinetics compared to free doxorubicin, with an increased distribution plasma half-life from 5 min to 1 h (26). The stable peptidyl linker also ensures that little or no free doxorubicin is liberated into the circulation following i.v. administration, thus increasing the therapeutic index of the conjugate. There is *in vitro* data in a human ovarian carcinoma cell line indicating that PK1 can overcome the P-glycoprotein cell surface membrane pump associated with the MDR phenotype (27).

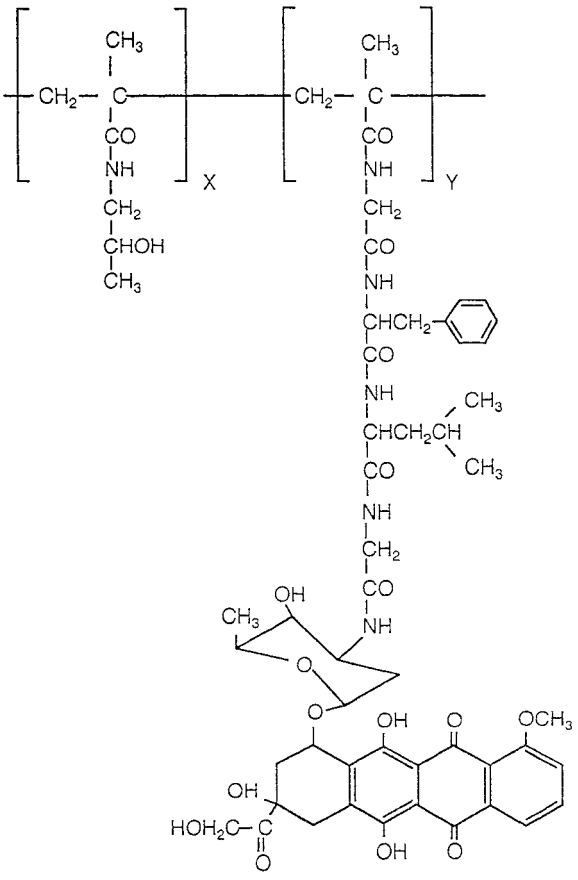

Fig. 1 Structure of PK1 (HPMA copolymer doxorubicin). The molecular weight of the compound is 28,000 [doxorubicin, 8.5% (w/w)].

In vivo antitumor activity of PK1 has been examined using a large panel of model tumors (Ref. 18 and references therein). i.p. administration of PK1 has been shown to display a higher activity profile than free doxorubicin against the ascitic tumor model L1210, melanoma B16F10, Walker sarcoma, P388 leukemia, M5076, and the human colon xenograft LS174T.

Animal toxicology studies (28) revealed that the single i.v. LD_{50} for male MFY mice was 63 mg/kg and that the MTD was extrapolated at 45 mg/kg. In multiple-dose studies (5 consecutive weekly i.v. injections) half-MTD doses (22.5 mg/kg) resulted in high mortality, indicating that the single dose study was inadequate for setting multiple-dose levels. The MTD for multiple-dose studies was 12 mg/kg. In multiple-dose studies using male Wistar rats, a dose of 5 mg/kg was established as the MTD. Following a single i.v. dose, hematological changes were observed in rats and mice soon after treatment (decreased WBCs, platelets, and erythrocytes). WBC changes persisted until d31 in both species, returning almost to normal by d56. Extramedullary hemopoiesis was observed, confirming that this myelosuppression was reversible. Weight loss was also noted in most animals, especially in multiple-dose studies, along with ambulatory problems, suggesting an effect on the nervous sys-

[3] The abbreviations used are: EPR, enhanced permeability and retention; CL, clearance; MDR, multidrug resistance; HPMA, *N*-(2-hydroxypropyl)methacrylamide; MTD, maximum tolerated dose; d, day; RNVG, radionucleotide ventriculography; CTC, Common Toxicity Criteria; LVEF, left ventricular ejection fraction; PR, partial response; NSCLC, non-small cell lung cancer.

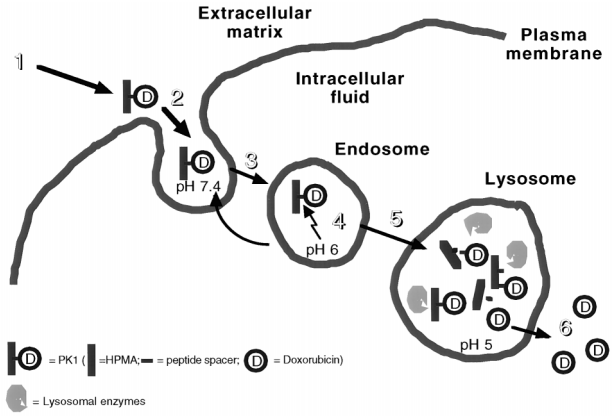

Fig. 2 Schematic representation of the principle of macromolecular uptake by the EPR mechanism and drug release intracellularly by cleavage of the peptidyl linker by lysosomal enzymes. Following pinocytic internalization, the macromolecule is transferred to the lysosomal compartment, where it is exposed to many lysosomal enzymes. Free drug is then liberated intracellularly, and the immunologically inert polymer is eventually excreted renally (or metabolized if biodegradable). *1*, increased vascular permeability and poor lymphatic drainage leads to selective retention of PK1 in tumors (EPR); *2*, internalization of PK1 by pinocytosis; *3*, transfer to endosomal compartment; *4*, hydrolysis of acid-sensitive spacers; *5*, transfer to lysosomal compartment; *6*, drug liberated intracellularly.

Table 1 Patient population

Characteristic	No. of patients
Total no. of patients	36
No. of males/no. of females	20/16
Mean age, yr (range)	58.3 (34–72)
Performance status	
0–1	31
2	5
Previous medical treatment for malignancy	
Chemotherapy and radiotherapy	12
Chemotherapy[a]	28
Radiotherapy	14
Hormones/immunomodulators	8
No prior therapy	6
Tumor type	
Colorectal	8
Adenocarcinoma, unknown primary[b]	5
Breast	3
Ovary	3
Biliary tract	3
Pancreas	3
Urinary tract	3
Head/neck	3
NSCLC	2
Mesothelioma	2
Stomach	1

[a] Six were treated previously with anthracyclines: 4 with doxorubicin (200, 120, 80, and 50 mg/m^2 cumulative doses) and 2 with epirubicin (160, 480 mg/m^2 cumulative doses).

[b] One patient had coexisting soft tissue sarcoma of the lower limb, treated with radiotherapy.

tem. Histologically, changes were observed in bone marrow, liver (rats only), gastrointestinal tract, thymus, and testes. Multiple-dose studies produced mild renal tubular changes without urinary abnormalities. In anesthetized beagle dogs at a dose of 8 mg/kg, acute but reversible cardiovascular changes (hypotension and a compensatory reflex tachycardia) were observed.[4] No fatalities occurred in these animals, and slower i.v. infusions abrogated these changes.

On the basis of these studies, a dose of 15 mg/m^2 (doxorubicin-equivalent) is equivalent to 1/10 of the LD_{10} calculated from the multiple-dose study data, and 20 mg/m^2, given as an i.v. infusion every 3 weeks, was chosen as a starting dose for the Phase I study.

PATIENTS AND METHODS

Patient Selection. Thirty-six patients were entered into the study, and their characteristics are listed in Table 1. All had histologically confirmed solid tumors that were refractory to conventional treatments, and all were between 34 and 72 years old. Fully informed written consent was obtained prior to drug administration. All patients had a performance status of 2 or better on the Eastern Cooperative Oncology Group-Zubrod-WHO scale and had a life expectancy of at least 12 weeks. Other eligibility criteria included the presence of adequate bone marrow, hepatic, and renal function, as evidenced by: neutrophil counts of ≥2,000/mm^3; platelet counts of ≥100,000 mm^3; hemoglobin levels of ≥10 g/liter; bilirubin levels of <20 μmol/liter; aspartate transaminase/alanine transaminase levels <2 times the upper limit of normal; and creatinine levels of <150 μmol/liter. Patients were eligible if they have received prior chemotherapy, but at least 4 weeks were required to have elapsed since administration (6 weeks for mitomycin and nitrosureas). Prior anthracyclines were allowed, providing that the cumulative dose of doxorubicin was <200 mg/m^2 (epirubicin dose of <450 mg/m^2) and that left ventricular function was within normal limits on either cardiac ultrasound or by RNVG scan. Any previous treatment with radiotherapy must not have involved >25% of red bone marrow, and at least 6 weeks were required to have elapsed. Patients were required to have no pretreatment peripheral neuropathy of more than CTC grade I and to be continent of urine and feces to safely conduct ^{131}I imaging. Patients of childbearing age were required to be using medically approved contraceptive precautions. Pregnant or lactating women were excluded.

All patients had a baseline history and physical examination, full blood count with differential, biochemical profile, urinalysis, chest X-ray, and 12-lead electrocardiogram. Qualitative assessment of myocardial function by ultrasound or quantitative measurement of LVEF by RNVG was carried out prior to the first course of chemotherapy. Tumor imaging was performed by appropriate radiological studies, including computer-assisted tomography and ultrasound, prior to the first course and every two cycles thereafter. All concomitant medication was documented throughout the study, and no other experimental drugs were administered. Neurological assessment was carried out before administration of study drug by a structured questionnaire and neurological examination (29).

[4] Pharmacia and Upjohn, data on file.

Table 2 Dose escalation scheme

Dose (mg/m^2 doxorubicin equivalent)	No. of patients	No. of courses/ patient	Median cumulative dose, mg/m^2 (range)
20	3	2, 1, 3	40 (20–60)
40	3	2, 1, 1	40 (40–80)
80	3	6, 2, 4	320 (160–480)
120	3	7, 4, 7	840 (480–840)
180	6	1, 6, 1, 7, 1, 1	180 (180–1260)
240	6	1, 4, 2, 4, 2, 4[a]	480 (240–960)
280	6	2, 3, 6, 1, 2, 3	700 (280–1680)
320	6	2, 2, 1, 1, 1, 2	480 (320–640)

[a] Courses 3 and 4 for this patient were at 280 mg/m^2.

During the study, patients were seen weekly in the outpatient clinic for clinical assessment, recording of all toxicities, and measurement of hematological and biochemical parameters. Repeat cardiac ultrasound or RNVG scans were carried out at cumulative doses equivalent to >200 mg/m^2 doxorubicin and every two courses thereafter or at completion of chemotherapy. Toxicities were graded according to the National Cancer Institute CTC. Standard response criteria were used for assessment (30). Neurological assessment was carried out 24 h after the first course and weekly thereafter. Following the first dose only, patients had their blood pressure and pulse rate recorded every 30 min for 4 h in total. Patients were taken off the study if their disease progressed, if they suffered unacceptable toxicity, or at their own request.

Administration. PK1 was supplied by Pharmacia and Upjohn as a freeze-dried lyophilized powder in glass vials containing 50 mg of doxorubicin-equivalent and ~530 mg of polymer [PK1 contains 8.5% (w/w) doxorubicin]. This was reconstituted with 25 ml of sterile 0.9% saline for injection to achieve final concentrations of 2 mg/ml. Reconstituted drug was then added to a sterile, nonpyrogenic bag (Clinitec; Baxter Healthcare Ltd, Norfolk, United Kingdom), for final administration. A peripheral vein was cannulated, and the drug was infused by volumetric pump at a rate of 4.16 ml/min. Once administration was complete, the line was allowed to flush through with saline for ~2 min. Patients were hospitalized for the first course only, receiving further courses of PK1 as an outpatient once every 3 weeks until withdrawal from the study. Dose escalation was based on clinical criteria, and a modified Fibonacci scheme was used (see Table 2). At each dose level, the first patient was observed for 3 weeks (or until recovery from acute toxicity) before a further two patients were entered at the same level. At least two of these three patients were observed for 3 weeks before patients were entered at the next dose level. In the presence of clinically significant toxicity (more than grade II neutropenia, gastrointestinal toxicity, hepatotoxicity, or renal toxicity or more than grade I thrombocytopenia, neurological toxicity, or cardiac toxicity), three more patients were entered at that dose level. Escalation was stopped once the MTD had been established. This was defined as the dose at which >30% of the patient population would suffer dose-limiting toxicity due to the drug. Such toxicity was defined as grade II neurotoxicity or grade III other nonhematological toxicity (excluding alopecia and nausea) or grade IV hematological toxicity, according to CTC criteria, that lasted >4 days or was associated with fever or bleeding.

Radionuclide Imaging Studies. The 21 patients who consented to this additional section of the study were pretreated with oral potassium iodate (170 mg/day) from d−1 to d7 to ensure full thyroid blockade following administration of ^{131}I-labeled PK1. The labeled material was prepared by adding 100 MBq of carrier-free [^{131}I]iodide to 32 mg of PK1 (containing ~2.6 mg of doxorubicin) in an iodogen reaction vial. Following the iodination reaction (~20 min later), free [^{131}I]iodide was removed by passing the material through a G-25 Sephadex column. Radiochemical purity was determined by TLC prior to patient administration. Mean (± SD) activity administered was 46.7 ± 2.3 MBq, with a mean radiochemical purity of 97.5 ± 1.4%. The labeled PK1 was administered by fast injection through the lateral entry port of a fast-flowing i.v. infusion of 0.9% saline, immediately following completion of PK1 infusion. Gamma camera imaging was then performed at ~2 and 24 h, with further imaging 8 days later. Organ/tumor uptake was quantified by methods described previously (31).

Pharmacokinetics. Blood samples were taken from a heparinized cannula in the opposite arm to the drug administration site. Ten-ml samples of blood were taken into heparinized tubes at predose, 15 min into infusion, at the end of infusion, and at the following times after infusion: 5, 10, 15, 30, and 60 min and 2, 4, 8, 12, and 24 h. Samples were collected up to 16 days postdose in patients who were hospitalized or were willing to travel to the hospital. Samples were immediately centrifuged, and the plasma removed was split into two equal aliquots and stored at −20°C until analysis. Urine collections were attempted at pretreatment, from 0 to 8 h and from 8 to 24 h in separate containers. Thirty-ml samples were collected from the pretreatment collection, and 40-ml samples were collected from the other containers and stored at −20°C until analysis. At the end of the collection time, the total volume of each collection interval was measured. Patients who had received ^{131}I-labeled PK1 had their blood and urine samples stored separately so that appropriate radiological protection measures were taken at analysis. All plasma and urine samples were shipped and quantitatively analyzed by the Bioanalytical Laboratory, Pharmacokinetics and Metabolism Department, Pharmacia and Upjohn (Nerviano, Italy).

The levels of free doxorubicin and polymer-bound doxorubicin and some of its possible metabolites released *in vivo* (*e.g.*, 13-dihydrodoxorubicin) were determined in plasma and urine using a fully validated high-performance liquid chromatography method with fluorescence detection (32). All analyses were performed to Good Laboratory Practice standards. The quantitation limits in plasma for free doxorubicin and bound doxorubicin were 0.38 and 5.10 ng/ml, respectively. Values for urine were 15.0 and 25.5 ng/ml, respectively. Correlation coefficients (r) for the regression were always >0.99. Briefly, free compounds were extracted twice with *i*-propanol-chloroform mixture (25:75, v/v), and the first extraction was performed at physiological pH and the second after buffering to pH 8.4, to extract the aglycones and the glycosides, respectively. Determination of total doxorubicin (free plus polymer-bound) was performed after quantitative acid hydrolysis to release doxorubicinone from free or polymer-bound doxorubicin by extraction

Table 3 Nausea and vomiting

Dose (mg/m^2)	No. of patients/dose level[a]	No. of evaluable cycles	Nausea grade				Vomiting grade				
			0	1	2	3	0	1	2	3	4
20	3	6	2	1	0	0	3	0	0	0	0
40	3	4	1	1	1	0	2	0	1	0	0
80	3	12	2	1	0	0	3	0	0	0	0
120	3	18	1	2	0	0	2	1	0	0	0
180	6	17	2	1	3	0	3	2	1	0	0
240	6	15	3	2	1	0	2	2	2	0	0
280	6	19	2	2	1	0	3	1	1	0	0
320	6	9	0	3	1	2	2	1	1	2	0

[a] One patient started at 240 mg/m^2 for two cycles and was subsequently dose-escalated to 280 mg/m^2 for a further two cycles.

Table 4 Hematological toxicity

Dose (mg/m^2 dox-eq)[a]	No. of patients/dose level	No. of evaluable cycles	Neutropenia grade					Thrombocytopenia grade					Anemia grade				
			0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
20	3	6	3	0	0	0	0	3	0	0	0	0	2	2	1	0	0
40	3	4	3	0	0	0	0	3	0	0	0	0	1	1	1	0	0
80	3	12	3	0	0	0	0	3	0	0	0	0	2	1	0	0	0
120	3	18	3	0	0	0	0	3	0	0	0	0	1	2	0	0	0
180	6	17	5	0	1	0	0	3	3	0	0	0	0	4	2	0	0
240	6	15	4	0	1	1	0	4	0	0	2	0	0	0	5	1	0
280	6 + 1[b]	19	2	2	1	2	0	4	3	0	0	0	1	3	2	0	1
320	6	9	1	0	2	0	3	2	3	1	0	0	0	3	3	0	0

[a] dox-eq, doxorubicin-equivalent.
[b] Includes two cycles from one patient who had previously received two cycles at 240 mg/m^2 and was dose-escalated.

with the above solvent mixture at pH 7.4. The organic phase was evaporated to dryness and then separated by high-performance liquid chromatography using a Nova-Pak C18 reverse-phase column and eluted with the mobile phase methanol-acetonitrile-phosphate buffer (pH 1.4; 0.01 M) mixture (10:25:65, v/v/v). Fluorescence detection was set at an excitation wavelength of 480 nm and an emission wavelength of 560 nm.

The pharmacokinetics of total and free doxorubicin were each determined using a population approach in which all concentrations were analyzed simultaneously using the package NONMEM (33). Preliminary analyses indicated that both total and free doxorubicin were best described by a biexponential disposition model, and interpatient variability on parameters was assumed to correspond to an exponential model. Additive, exponential, and combined additive and exponential error models were compared for their ability to describe residual error on concentration measurements.

Following the population analysis, individual estimates of CL volume of the central compartment (*V1*), volume of the peripheral compartment (*V2*), and intercompartmental CL (*Q*) were estimated by NONMEM. Volume of distribution at steady state (V_{ss}), distribution half-life, and elimination half-life were derived from these parameters using standard equations.

Scatterplots were prepared of CL and V_{ss} against the available clinical covariates, age, weight, body surface area, biochemical measurements, and so on and examined for obvious trends. Factors expected to influence CL and V_{ss} and factors identified from the scatterplots as potentially influencing these parameters were then included in the population model. Hierarchical models were statistically compared by a likelihood ratio test, based on the difference in objective function value. Significance was set at $P < 0.005$ (a difference of >7.9 with 1 degree of freedom). Models were also compared on the basis of residual and weighted residual plots and SEs of the parameter estimates.

RESULTS

Toxicity. Thirty-six patients received a total of 100 courses of PK1, and all were evaluable for toxicity. The numbers of patients and courses administered and the total cumulative doses (doxorubicin-equivalent) are summarized in Table 2. Nausea and vomiting are summarized in Table 3. Hematological events observed during the study are shown in Tables 4 and 5. Lethargy, mucositis, and hepatic toxicities are shown in Table 6. One patient treated for two cycles at 240 mg/m^2 had two subsequent cycles at an escalated dose of 280 mg/m^2 because no significant toxicities had been encountered at the lower dose level at that point in the study. This patient's toxicities are analyzed according to the dose received for the particular cycle and have been tabulated accordingly. No other intrapatient dose escalations were performed.

Nausea was observed at all levels, vomiting was observed at all levels of $\geq$40 mg/m^2, and both appeared to be dose related. Generally, the onset of emesis was within the first 24 h from treatment and was occasionally prolonged for 7–14 days. All patients experienced nausea on repeated dosing, but it was generally mild (grade I). Prophylactic antiemetics were not routinely given at dose levels of $<$280 mg/m^2, although patients

Table 5 Median and range of neutrophil count nadir ($\times 10^9$/liter) and time to nadir (days)

Dose (mg/m^2 dox-eq)[a]	No. of patients/ dose level	No. of evaluable cycles	Median neutrophil nadir (range)	Median time to nadir (range)
20	3	6	5.9 (2.9–7.5)	15 (8–21)
40	3	4	4.9 (2.6–6.1)	14 (7–21)
80	3	12	3.71 (2.9–6.1)	21 (1–21)
120	3	18	6.87 (3.3–9.5)	21 (1–21)
180	6	17	3.3 (1.0–5.4)	15 (15–21)
240	6	15	2.2 (0.6–3.7)	15 (15–21)
280	6	19[b]	1.8 (0.5–3.4)	15 (7–21)
320	6	9[c]	1.4 (0.02–5.42)	15 (15–21)

[a] dox-eq, doxorubicin-equivalent.
[b] Includes two cycles from patient initially treated at 240 mg/m^2 for two cycles and then dose-escalated. One cycle was only to d 15.
[c] One cycle was only to d15. Three of nine cycles at this level were associated with febrile neutropenia (see text).

were given a supply of domperidone tablets (Motilium, 20 mg every 6 h) to take if required. Severe nausea and/or vomiting (CTC grade III/IV) was not observed except at levels of $\geq$240 mg/m^2. At dose level 320 mg/m^2, it became apparent that emesis was becoming a significant problem. Two of the first 4 patients experienced severe emesis (nausea and/or vomiting CTC grade III), and therefore, the final two patients at this level were treated prophylactically with i.v. 5-HT$_3$ antagonists (3 mg of granisetron; Kytril; Smith-Kline Beecham) and 8 mg of i.v. dexamethasone prechemotherapy, followed by oral domperidone (doses as above) for 7 days. Neither of these patients experienced nausea or vomiting that was more severe than grade I. Thereafter, the final cohort of patients (treated at 280 mg/m^2) were routinely prescribed prophylactic i.v. 5-HT$_3$ antagonists (3 mg of granisetron; Kytril; Smith-Kline Beecham) and 8 mg of i.v. dexamethasone together with oral domperidone. Subsequently, none of the patients at this dose level experienced significant (more than grade II) drug-related nausea and vomiting.

Loss of appetite was commonly present at baseline, and many patients developed CTC grade I anorexia following PK1 administration, in association with nausea. However, one patient developed CTC grade III anorexia, concurrent with grade III nausea and vomiting.

Hematological events were observed at all dose levels, but at $<$180 mg/m^2, only anemia was apparent; the majority of the anemia observed was preexisting CTC grade I/II and probably due to underlying malignancy and other baseline conditions. CTC grades for neutrophil, platelets, and erythrocyte events following PK1 administration are shown in Table 4.

Thrombocytopenia was not dose related and was seen at significant levels (CTC grade III) in only two patients at 240 mg/m^2.

The median nadir neutrophil counts at each dose level are summarized in Table 5 and clearly show a dose relationship between levels 180 and 320 mg/m^2. Of the six patients receiving the top dose level of 320 mg/m^2, three had received prior chemotherapy, but none had received significantly myelosuppressive regimens. There was no statistically significant association observed between previous chemotherapy and degree of PK1-induced myelosuppression at any dose level.

Three patients treated at 320 mg/m^2 developed fever associated with CTC grade IV neutropenia. A 57-year-old male with colorectal cancer metastatic to liver was admitted on d8 of his second cycle with grade IV neutropenia, grade III mucositis, and a fever. i.v. antibiotics and oral antifungals were started, and the patient recovered without sequelae. Blood and stool cultures were negative. A 51-year-old man with pancreatic cancer metastatic to liver was admitted on d14 of his second cycle with febrile grade IV neutropenia, a chest infection, and oropharyngeal thrush. i.v. antibiotic therapy was again initiated plus oral antifungal agents, and his temperature and condition improved. He was noted retrospectively to have had elevated hepatic aminotransferases (CTC grade II), serum bilirubin (CTC grade II), and alkaline phosphatase (CTC grade III) on d1 of the cycle, and these levels were shown to have increased (bilirubin grade IV, aminotransferases grade III) by d14, indicating possible hepatic toxicity. His grade IV neutropenia lasted for 4 days, and although his aminotransferases had returned to baseline values by d21, the bilirubin and alkaline phosphatase levels continued to rise. A repeat ultrasound scan confirmed that his disease was, in fact, progressing, and he did not receive further chemotherapy. A 60-year-old male with colorectal cancer and hepatic metastases had CTC grade IV neutropenia on d16 of cycle 1 and CTC grade II fever on d17. There was no clinically obvious focus of infection, and no antibiotics were prescribed. The fever resolved spontaneously within 24 h. Retrospective biochemical analysis showed that his bilirubin levels had increased to CTC grade III on d8 but were in decline during the neutrophil nadir. Subsequent progression of the hepatic metastases was noted, and no further chemotherapy was administered. Finally, a 62-year-old male with colorectal cancer and hepatic metastases became febrile (CTC grade II) on d8 of cycle 2 of PK1 at 280 mg/m^2. Although not neutropenic, he was commenced on i.v. antibiotics to cover suspected infection of his Hickman line (indwelling subclavian double lumen catheter). The fever spiked intermittently for 72 h before resolution, and the patient was asymptomatic during this time. Although other infections were recorded during the study (*e.g.*, oropharyngeal candidiasis and urinary tract infection), they were not considered serious.

One patient at 320 mg/m^2 had his second cycle of PK1 delayed for 7 days due to a low (CTC grade II) neutrophil count on d21 of cycle 1. Even at the higher dose levels (240, 280, and 320 mg/m^2), there was no evidence of cumulative or prolonged severe neutropenia.

There was one death during the study. A 68-year-old male with extensive locally advanced squamous carcinoma of the maxillary antrum suddenly became unwell on d15 of his second cycle of PK1 at 280 mg/m^2. He was noted to have CTC grade III neutropenia with a normal platelet count, and was afebrile. He became acutely confused immediately following his outpatient visit and collapsed. Clinical examination revealed central cyanosis with a Cheyne-Stokes respiration pattern and bilateral up-going plantars (positive Babinski sign) without any other localizing neurological signs. Before any investigations could be initiated, he died. Postmortem examination was refused. Because clinical evidence of disease progression was evident at the time, cause of death was considered to be due to tumor expansion and bony erosion within the skull, with subsequent intracerebral hemorrhage.

Table 6 Lethargy, mucositis, and hepatic toxicity following PK1 administration

Dose (mg/m^2 dox-eq)[a]	No. of patients	No. of evaluable cycles	Maximum CTC toxicity/patient									
			Lethargy grade			Hepatic grade				Mucositis grade		
			1	2	3	1	2	3	4	1	2	3
20	3	6	0	0	0	0	0	0	0	0	0	0
40	3	4	0	1	0	0	0	0	0	0	0	0
80	3	12	1	0	0	0	0	0	0	1	0	0
120	3	18	1	1	0	1	0	0	0	0	0	0
180	6	17	3	0	0	1	0	0	0	1	1	0
240	6	15	0	1	1	1	0	0	0	0	3	0
280	6	19[b]	2	3	0	2	1	0	0	1	3	0
320	6	9	0	1	0	0	1	1	1	1	2	2

[a] dox-eq, doxorubicin-equivalent.
[b] Includes two cycles from a patient already treated with two cycles at 240 mg/m^2 and escalated.

Significant (more than or equal to CTC grade II/III) mucositis was seen at all levels of ≥180 mg/m^2 and appeared to be dose related (Table 6). The appearance of higher grades of mucositis was not related to the cumulative number of cycles of chemotherapy received. Oral ulceration (CTC grade II) tended to appear at around d7–d15 and had usually resolved to at least grade I by the time of the next treatment cycle. At 320 mg/m^2, two of six patients experienced CTC grade III mucositis.

Neurological toxicity was noted at dose levels of 120 and 180 mg/m^2 and was generally manifest as CTC grade I peripheral paraesthesia (9 of 36 patients; 25%). However, one patient at 180 mg/m^2, a 63-year-old female with locally recurrent breast cancer, developed sudden onset of dizziness and lack of coordination on d20 of cycle 1. Neurological examination revealed a positive Romberg's sign and an inability to walk heel-toe without falling to the right. General hyperreflexia was noted, but plantars were down-going. No other neurological signs (long tract or cerebellar) were present, and fundoscopy was unremarkable. Symptoms and signs subsequently resolved completely within 7 days. A magnetic resonance imaging brain scan revealed a small area of infarction in the left posterior parietal lobe, but no evidence of metastatic disease. A repeated magnetic resonance imaging scan 2 months later confirmed the presence of cerebral infarction. This feature was not considered secondary to PK1 administration. However, no subsequent cycles of PK1 were administered, due to progressive disease occurring during the period of investigation.

Evidence for hepatic toxicity (defined as a reversible elevation in hepatic aminotransferases alanine aminotransferase and/or aspartate aminotransferase or serum bilirubin following PK1 chemotherapy in the absence of disease progression) was present at all dose levels of ≥120 mg/m^2 (Table 6). In all, 9 of 36 patients (25%) developed changes in liver function tests during 19 of 100 cycles (19%) of PK1, and this appeared to be dose related. The principal abnormalities noted were reversible elevation of aminotransferases (9 of 9; 100% affected patients), which occurred at variable time points following chemotherapy (range, d4–d21), and to a lesser extent, hyperbilirubinemia (2 of 9 or 22% affected patients). Of the nine patients demonstrating biochemical evidence of hepatic disturbance, six (67%) had preexisting liver metastases. At the MTD, two of six patients had grade III or more aminotransferases and/or bilirubin elevations. Both these patients had preexisting liver metastases and have been described above. Progressive disease was noted in both patients soon after the development of deranged liver function tests and may, therefore, have contributed to the degree of biochemical derangement observed.

The mean cumulative dose of PK1 (doxorubin-equivalent mg/m^2) administered to all patients was 508 mg/m^2 (range, 20–1680 mg/m^2. There were no clinically significant dysrhythmias or episodes of congestive cardiac failure. Six patients had received previous anthracyclines (Adriamycin or epirubicin). All 36 patients had either baseline qualitative estimates of cardiac function by echocardiography or quantitative estimates of LVEF by RNVG, but because of nonattendance (due to death or clinical deterioration with progressive disease), only nine (25%) patients had follow-up examinations. These nine patients received a mean cumulative dose of PK1 (doxorubicin-equivalent) of 782 mg/m^2 (range, 320-1140 mg/m^2; and one had also previously received 200 mg/m^2) of doxorubicin (making a total cumulative doxorubicin-equivalent dose of 1040 mg/m^2). One patient, a 63-year-old male with transitional cell bladder carcinoma metastatic to lungs and mediastinum, demonstrated a clinically significant drop in LVEF from a baseline 47% to 30%, with the qualitative appearance of septal and apical hypokinesia, following four cycles of PK1 (cumulative doxorubicin-equivalent dose 1040 mg/m^2). At this stage, his known pulmonary disease was stable radiologically, but his general condition had deteriorated, with symptoms of cachexia and recurrent hematuria with iron-deficiency anemia (hemoglobin, 6.0 g/liter). Despite this apparent drop in LVEF, no features of congestive cardiac failure were evident, and his overall deterioration was thought to be due to progression of his disease. None of the other patients had any evidence of qualitative/quantitative changes in cardiac function.

Alopecia occurred in 30% (11 of 36) patients and was generally mild (CTC grade I). It was only seen at doses of ≥180 mg/m^2, with grade II hair loss present in only three patients (8%) after a mean cumulative PK1 dose of 547 mg/m^2.

Two patients developed dermatological toxicities during the study. One patient at 240 mg/m^2 developed small blisters, in association with preexisting varicose eczema, on the lateral malleoli of both ankles at around the time of the hematological nadir. These were bacteriologically confirmed to be due to staphylococcal infection and disappeared with hematological recovery. Another patient developed a bullous eruption over the

Table 7 Mean biological uptake (% dose ± SD) in various organs and tumor tissue

Time	Left kidney	Right kidney	Heart	Liver	Tumor[a]
2–3 h	1.26 ± 0.57	1.27 ± 0.6	2.68 ± 1.2	2.52 ± 1.58	2.2 ± 2.1
24 h	0.99 ± 0.41	1.19 ± 0.52	0.84 ± 0.47	1.59 ± 0.82	1.3 ± 0.4
d 7	0.51 ± 0.26	0.56 ± 0.3	0.33 ± 0.36	1.62 ± 0.67	0.5 ± 0.3

[a] Only six patients were found to have identifiable tumor uptake.

sacrum following cycle 1 of PK1, which subsequently faded during the following three cycles. This was considered to most likely to represent a hypersensitivity reaction to the potassium iodate tablets given as part of the imaging protocol on the first cycle.

Significant lethargy (Table 6) was reported by 14 patients (39%) and was observed more frequently at the higher dose levels and often on later cycles of PK1.

Responses. Two PRs and 2 minor responses were documented in four patients. A 47-year-old male with NSCLC previously treated with radiotherapy demonstrated a CR in liver metastases after four cycles at 120 mg/m^2 and a PR in axillary lymphadenopathy after two cycles. He remained in overall PR for 16.7 weeks. This response was not confirmed independently, because the evaluation was by ultrasound. A 49-year-old woman with NSCLC that was previously unresponsive to chemotherapy (mitomycin, ifosfamide, and cisplatin) demonstrated a CR in her measurable cervical lymphadenopathy after four cycles at 180 mg/m^2. Her primary tumor was nonmeasurable but improved on serial chest radiology. Her overall PR lasted for 10.6 weeks. Two minor responses (not achieving the strict criteria of a PR) were also observed. A 65-year-old man with metastatic colorectal cancer previously shown to be unresponsive to a weekly 5-fluorouracil/leucovorin regimen demonstrated a >50% volume reduction in one of his two measurable liver metastases following four cycles of PK1 at 280 mg/m^2. The other liver metastasis and all pulmonary metastases remained static in size, and the overall response duration was 7 weeks. Finally, a 34-year-old woman with heavily pretreated breast cancer and a nonresponder to prior anthracycline therapy demonstrated improvement of her chest wall disease after two cycles of PK1 at 80 mg/m^2. Most of these lesions were measurable prior to commencement of chemotherapy (the largest being 16 × 16 mm), and all flattened and became only diffusely palpable during treatment. She was taken off study after six cycles at her own request.

Radionuclide Imaging Studies. The liver and kidneys and also the heart, to a lesser extent, could nearly always be identified at each imaging session. The urinary bladder was identifiable at 2–3 and 24 h. Biological uptake values for the kidneys, liver and heart are shown in Table 7. In 5 of 21 patients, there was uptake (<0.6% dose) in the right inferior quadrant at 24 h, thought to be due to a small amount of hepatobiliary excretion of the labeled material. In 6 of 21 patients, there was uptake at known tumor sites, but it was not always identifiable at each imaging session. Fig. 3 shows the 2-h head image of a patient with head and neck cancer and demonstrates increased uptake at the primary tumor site on the left side of the neck. Mean biological uptake values (percentage dose ± SD) in the six patients with tumor uptake were 2.2 ± 2.1 at 2–3 h, 1.3 ± 0.4 at 24 h, and 0.5 ± 0.3 at 8 days. From the total trunk counts and using knowledge of the gamma camera response to known amounts of ^{131}I in the body, we were able to obtain some indication of whole-body turnover of the labeled material. The disappearance curve appears to be at least biphasic, with 55% being cleared with a biological half-life of 3.7 h and 45% being cleared with a biological half-life of 9 days.

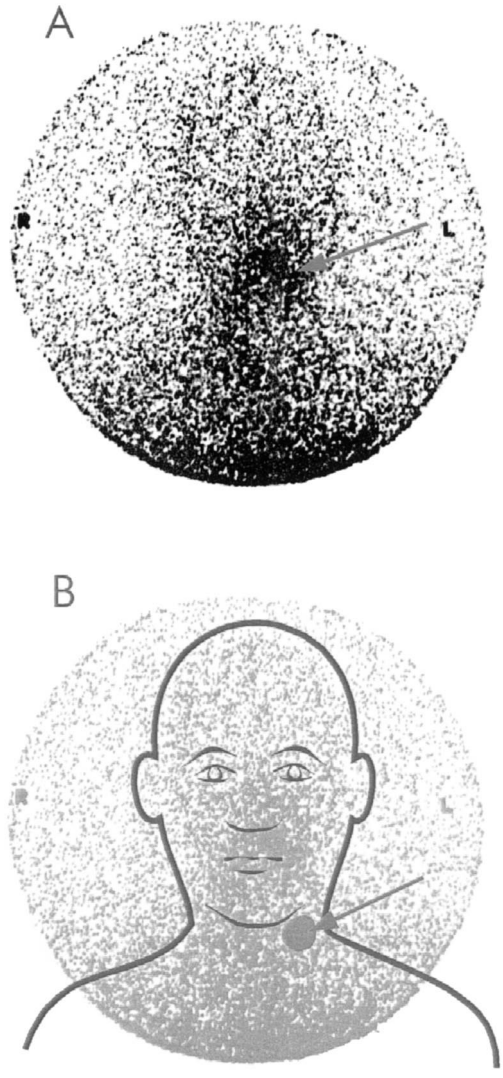

Fig. 3 Two h posttreatment, gamma camera head image (*A*) of a patient with a large fungating carcinoma of the left cervical region demonstrating increased uptake with radiolabeled drug at the tumor site (*arrow*); depicted by drawing in *B*.

Table 8 Population pharmacokinetic parameter estimates of PK1 and doxorubicin

	Clearance (liters/h)	Central volume (liters)	Intercompartmental clearance (liters/h)	Peripheral volume (liters)
PK1	0.174	4.55	0.72	9.57
Interindividual CV[a]	26%	40%	74%	76%
Free doxorubicin	179	1,340	8,310	16,900
Interindividual CV	35%	99%	159%	74%
Residual error				
PK1[b]	12–143%			
doxorubicin	43%			

[a] CV, coefficient of variation.
[b] CV varied from 143% at the lowest concentration (900 ng/ml) to 12% at the highest (154,000 ng/ml).

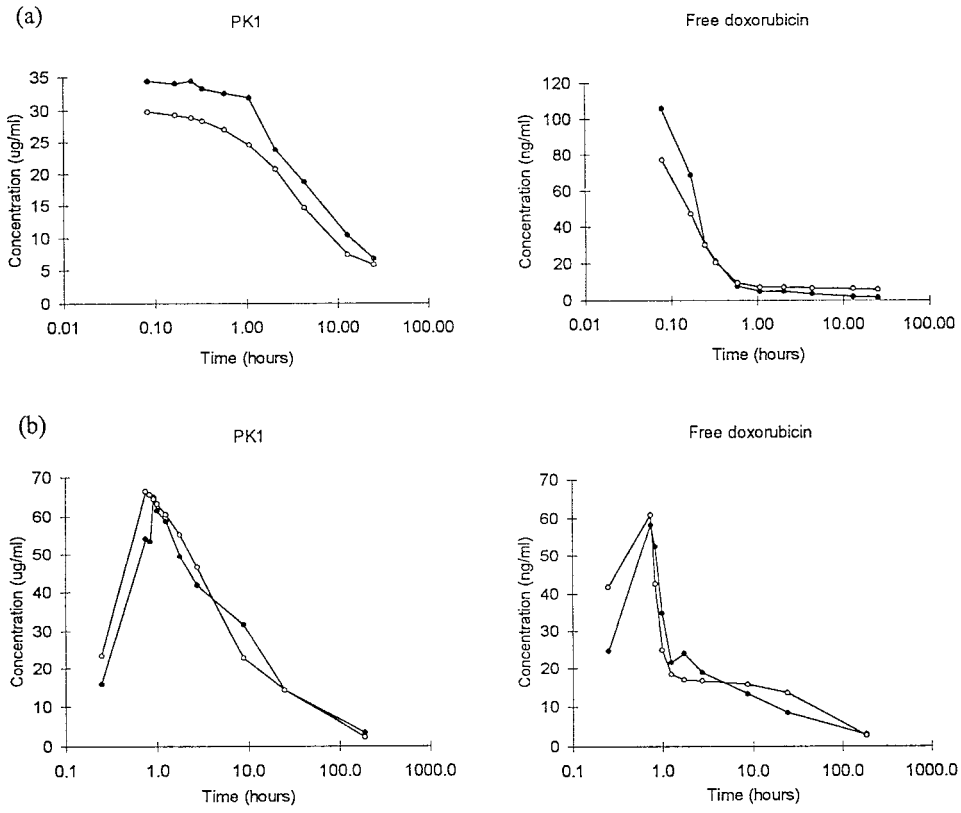

Fig. 4 Measured (●) and population-predicted (○) concentrations of PK1 and free doxorubicin (correlation coefficient, −0.44) for two patients receiving PK1 at 80 mg/m^2 (*a*) and 180 mg/m^2 (*b*). Time (*X axis*) is presented on a log scale for clarity.

Pharmacokinetics/Pharmacodynamics. Concentration data were available from 33 patients and comprised 388 total doxorubicin measurements (range, 8–15 per patient; median, 11 per patient) and 379 free doxorubicin measurements (range, 6–15 per patient; median, 11 per patient). Thirty-three concentrations of both free and total doxorubicin were measurable in 16 patients beyond 90 h after the start of the infusion. Urine samples were obtained from 20 patients, and although free doxorubicin was found to be present in urine (generally at low levels, *i.e.*, <5% doxorubicin-equivalent administered), samples were considered to be of insufficient quality to perform any meaningful analysis.

Total doxorubicin data were best described using a combined residual error model structure, whereas an exponential model was sufficient to describe the free doxorubicin data. Population average parameter estimates for total and free doxorubicin are presented in Table 8, and examples of pharmacokinetic profiles are shown in Fig. 4.

Total doxorubicin CL averaged 0.174 liter/h and individual estimates ranged from 0.145 to 0.279 liter/h. V_{ss} averaged 14.1 liters and ranged from 7.1 to 115 liters. Derived distribution half-life had a population average estimate of 1.8 h, and elimination half-life was 93 h. No obvious trends were apparent in scatterplots of PK1 CL and V_{ss} against patients' clinical characteristics, and there was no evidence of dose dependency in CL. When included as factors in the population model, weight, body surface area,and sex had no influence on the CL, *V1*, or *V2* of total doxorubicin, but dose had a small effect on *V1*. Free doxorubicin CL at 179 liters/h was 1000 times the CL of total doxorubicin, reflecting the low concentrations measured. Indi-

vidual estimates ranged from 110 to 276 liters/h, but due to the lack of concentration data beyond the distribution phase, estimates for patients on low doses (20–40 mg/m^2 tended to reflect the population mean values. V_{ss} had a derived population average estimate of 18,240 liters (range, 2,580–37,618 liters), distribution half-life was 0.07 h (range, 0.04–1.3 h), and elimination half-life was 108 h (range 13–228 h). No obvious important clinical covariates were identified from scatter plots, but the inclusion of dose as a covariable on *V1* and *V2* and height as a covariable on *V2* produced small improvements in the population model fit.

In summary, there was no evidence of dose dependency in CL, and no clinical factors were identified that strongly influenced the CLs of total or free doxorubicin. Finally, the toxicity data were examined with the aim of ascertaining the presence or absence of any pharmacodynamic relationship. Although patient numbers were small, there appeared to be an association between high concentrations of total doxorubicin (dose levels of $>$180 mg/m^2) and both myelosuppression and mucositis.

DISCUSSION

Here, we report the first clinical experience with the polymeric anthracycline conjugate, PK1, and confirm the promise shown in the preclinical studies that cytotoxic drug conjugation can decrease nonspecific organ toxicity, while at least maintaining antitumor activity.

The dose-limiting toxicities in this study were febrile neutropenia and mucositis. At the MTD, 320 mg/m^2, three of six patients developed fever and grade IV neutropenia, and two of six developed grade III mucositis. All three patients developing febrile neutropenia had hepatic metastases, and of the nine patients that developed disturbances in their liver enzymes following drug administration, 67% also had preexisting liver metastases, indicating a possible influence of hepatic function on drug metabolism. Despite the significantly increased doses of doxorubicin-equivalent administered to each patient (mean cumulative dose, 508 mg/m^2; range, 20–1680 mg/m^2), other anthracycline-like toxicities were markedly attenuated. Nausea and vomiting was not a problem with prophylactic 5-HT$_3$ antiemetic administration; only 8% patients experienced grade II alopecia; and there were no incidences of congestive cardiac failure, despite individual cumulative doses of doxorubicin-equivalence up to 1680 mg/m^2. However, it should be noted that, because only 25% patients received follow-up cardiac evaluation by ultrasound/RNVG, continued noninvasive monitoring of cardiac function should be a feature of Phase II trials.

The pharmacokinetic analysis used a population approach to analyze total and free doxorubicin data because full concentration-time profiles were not available for all individuals, particularly at the lower doses. Application of the population approach thus enabled data to be used from all patients.

The PK1 data were best described by a biexponential disposition model, and in most cases, the population mean parameter estimates provided good predictions of the measured concentrations. CL was well defined, and the interindividual variability was low, at 26%. In contrast, interpatient variability in intercompartmental CL and volume of the peripheral compartment were much higher, at 76 and 74%, respectively.

CL estimates obtained from the free doxorubicin data were 1000 times higher than the PK1 estimates (population mean CL estimate, 179 compared to 0.174 liters/h), reflecting the 1000-fold difference in concentrations. One patient, following administration of 320 mg/m^2 PK1, had unexpectedly low concentrations of total doxorubicin and high concentrations of free doxorubicin. These differences were apparent from the first sample, maintained throughout the profile, and associated with high estimates of total doxorubicin V_{ss} (115 liters) but low estimates of free doxorubicin V_{ss} (2580 liters). This patient subsequently experienced grade III nausea and grade IV vomiting, the worst at this dose level, but had not received prophylactic antiemetics.

Free doxorubicin had a very rapid distribution phase with a half-life of 5 min, whereas the distribution of total doxorubicin was slower, with a median estimate of 1.8 h. Average elimination half-lives were similar (93 h for total doxorubicin and 108 h for doxorubicin), but the correlation between individual elimination half-lives was not high (correlation coefficient, 0.44). These observations, coupled to the detection of doxorubicin at the first sample time (5–15 min after the start of the infusion) and the highly variable ratio, suggest that free doxorubicin was present in the formulation to varying degrees at the time of administration. The shape of the doxorubicin profile suggested that free doxorubicin was not being leached from cells after intracellular cleavage. If that were the case, a gradual increase in concentration might have been expected rather than simply a decline. However, such a declining profile could also be explained by the rate of final doxorubicin elimination being greater than the rate of intracellular free doxorubicin liberation by lysosomal enzymic action.

The lack of any obvious relationship between patients' clinical characteristics and pharmacokinetic parameters was not unexpected because all of the patients had essentially normal renal and hepatic function. It is noteworthy, however, that weight and body surface area had no effect on either CL or V_{ss}, despite their relatively wide ranges (41–103 kg and 1.2–2.2 m^2, respectively). The only relationships that could be identified were a positive linear relationship between *V1* and dose for both total and free doxorubicin, and a relationship between V2, dose, and height for free doxorubicin. It is unlikely that these apparent effects have any major clinical significance.

The imaging studies performed here have demonstrated that radioactively labeled drug can accumulate in some tumors, supporting a mechanism for preferential tumor macromolecular uptake. However, the disparity of uptake observed (only 6 of 21 patients) implies that this technique is suboptimal and produces a high proportion of false-negative results. One possible explanation is that, using this technique, the relatively low tumor uptake is essentially unidentifiable against a background of high organ uptake, *e.g.*, abdomen. For future trials, the imaging studies will be further refined, using both gamma camera and PET scanning.

Comparisons with liposomal drug delivery systems (*e.g.*, daunorubicin encapsulated by distearoylphosphatidylcholine/cholesterol, DaunoXome; and polyethylene glycol-coated liposomal encapsulation of doxorubicin, Doxil) are inevitable, because these have been extensively investigated as carriers for anticancer agents. However, there is a clear conceptual difference between these supramolecular systems, which are generally viewed as pharmaceutical formulations, and polymer con-

jugates, which are covalently bound to drugs and thus should be considered as new chemical entities. The mode by which tumors take up liposomal formulations is not completely understood but is likely to be EPR (34). However, these liposomes are not taken up by cells via pinocytosis but probably release encapsulated drug extracellularly, which subsequently diffuses into the cell, in marked contrast to the uptake mechanism of polymeric systems. Both DaunoXome and Doxil have longer half-lives, higher areas under the concentration-time curve, and lower CLs than either free daunorubicin or doxorubicin (35, 36). These compounds also appear to be free of significant cardiotoxicity and, generally, have decreased anthracycline-like toxicities. However, myelosuppression is still an important feature of both formulations at recommended doses (DaunoXome, 40–60 mg/m^2; Doxil, 25–40 mg/m^2), with neutropenia occurring at grade III/IV in 50–60% patients (35, 37). In addition, plantar-palmar erythrodysesthesia was noted to be dose limiting at higher cumulative doses of Doxil (38). Liposomal drug delivery systems have been found to be especially useful in the treatment of AIDS-associated Kaposi's sarcoma (35, 37) because conventional treatment with anthracyclines tends to be limited by cumulative toxicities (especially cardiac), preventing prolonged courses of chemotherapy. It is likely that the reason for the significant antitumor activity seen in AIDS-associated Kaposi's sarcoma is due to preferential macromolecular access via EPR. Histologically, these tumors show extensive but defective neovascularization of the surrounding extracellular matrix, with the derived vascular endothelium being discontinuous and full of holes. This provides an ideal environment for macromolecular capture and suggests that other treatments using the EPR mechanism, such as polymeric conjugates, would also show activity against such tumors.

In summary, PK1 has been shown to be active in refractory cancers, demonstrating no polymer-related toxicities. The recommended dose is 280 mg/m^2, given by i.v. infusion every 3 weeks, and this represents a dramatic increase in administered doxorubicin equivalence without the expected dose-limiting systemic toxicities at this dose. Clearly, Phase II evaluation is required to confirm this observed decrease in toxicity with maintained antitumor efficacy, and studies are planned in colorectal cancer, NSCLC, and breast cancer (anthracycline-resistant and anthracycline-naive). Interestingly, new *in vivo* experiments suggest that this schedule may not be optimal, and that a 3–5-day intermittent, lower=dose schedule leads to higher intratumor accumulation without increased toxicity.

A further development in polymer therapeutics is the identification of cell-specific receptors, which would theoretically provide a more effective way to attain organ- and tumor-specific targeting of the polymers. HPMA copolymer-doxorubicin-galactose (PK2, FCE 28069) has been designed to target the liver using the hepatocyte glycoprotein receptor to promote liver specific receptor-mediated uptake (39) and may, therefore, be useful in the treatment of primary or metastatic liver cancer. A Phase I clinical trial with this agent is underway. In addition, there are newer anthracycline analogues being synthesized, which are either more potent or less toxic than the parent compounds. One such analogue, methoxymorpholino-doxorubicin, is >80 times more potent than doxorubicin, is not subject to MDR (40), and has shown activity in refractory cancers in a relatively large Phase I clinical trial (41). An HPMA copolymer conjugate containing methoxymorpholino-doxorubicin (PNU 152243) has been developed (42) and, with subsequent improved tumor localization via EPR, could potentially increase the therapeutic ratio of this compound substantially. Also, an HPMA copolymer conjugate containing Paclitaxel has been described recently.

This clinical study provides a clinical "proof of principle" for polymeric constructs and demonstrates that polymers could provide an ideal platform for delivery of a range of antitumor compounds previously shown to be unacceptably toxic. Indeed, the EPR uptake mechanism, pinocytotic cellular uptake of drug-carrier construct, and subsequent controlled release of active drug intracellularly provides an ideal way of producing tumor cell-selective delivery of anticancer agents. Furthermore, polymeric constructs can potentially be used to deliver novel agents (*e.g.*, antiangiogenic) or other anticancer therapies [*e.g.*, oligonucleotides, gene therapies, and signal transduction modifiers; for review see Duncan *et al.* (43)]. Research is ongoing to identify novel polymeric carriers that, unlike HPMA, do not have the limitation of nonbiodegradability and subsequent requirement to be excreted renally. In addition, advances in imaging techniques (gamma camera imaging, PET scanning) should make it possible to identify and understand which tumors are more likely to be susceptible to the EPR approach.

This clinical trial demonstrates that conjugation of a conventional cytotoxic agent with a polymeric drug carrier decreases important dose-limiting toxicities and allows active drug to be delivered intracellularly, while maintaining antitumor activity. Clearly, the success of PK1 gives added impetus to future developments in this field. The ability to design different carriers, with various linkers, for a variety of anticancer agents, if clinically validated, would enable "designer" drugs, specific for individual tumor targets, to be produced. This, therefore, represents a novel and exciting approach in the fight against cancer.

ACKNOWLEDGMENTS

We would acknowledge the following individuals who contributed to this study in various ways: L. Adams, S. Tytler, C. Cameron, L. Light, C. Pagonis, M. Rocchetti, and L. Gumbrell.

REFERENCES

1. Benjamin, R. S. Pharmacokinetics of Adriamycin in patients with sarcomas. Cancer Chemother. Rep., *58:* 271–273, 1974.

2. Newell, D. R. Pharmacokinetic determinants of activity and toxicity of antitumour agents. Cancer Surv., *8:* 557–603, 1989.

3. Cassidy, J., Newell, D. R., Wedge S. R., and Cummings, J. Pharmacokinetics of high molecular weight agents. Cancer Surv., *17:* 315–341, 1993.

4. Duncan, R., Dimitrijevic, S., and Evagorou, E. G. The role of polymer conjugates in the diagnosis and treatment of cancer. STP Pharm. Sci., *6:* 237–263, 1996.

5. DelaFlor-Weiss, E., Uziely, B., and Muggia, F. M. Protracted drug infusions in cancer treatment: An appraisal of 5-fluorouracil, doxorubicin and platinums. Ann. Oncol., *4:* 723–733, 1993.

6. Trouet, A., and Joules, G. Targeting of daunomycin by association with DNA or proteins: a review. Semin. Oncol., *64:* 64–72, 1984.

7. Schecter, B., Neumann, A., and Wilchek, M. Soluble polymers as carriers of cisplatinum. J. Controlled Release, *10:* 75–87, 1989.

8. Levi-Schaffer, F., Bernstein, A., and Mesnorer, A. Reduced toxicity of daunorubicin by conjugation to dextran. Cancer Treat. Rep., *6:* 107–114, 1982.

9. Trouet, A., Masquelier, M., and Baurain, R. A covalent linkage between daunorubicin and proteins that is stable in serum and reversible by lysosomal hydrolases, as required for lysosomotropic drug carrier conjugate: *in vitro* and *in vivo* studies. Proc. Natl. Acad. Sci. USA, *79:* 626–629, 1982.

10. Seymour, L. W. Passive tumour targeting of soluble macromolecules and drug conjugates. CRC Crit. Rev. Ther. Drug Carrier Syst., *9:* 135–187, 1992.

11. Matsumura, Y., and Maeda, H. A new concept for macromolecular therapeutics in cancer chemotherapy; mechanism of tumoritropic accumulation of proteins and the antitumor agent SMANCS. Cancer Res., *6:* 6387–6392, 1987.

12. Maeda, H., and Matsumura, Y. Tumouritropic and lymphotropic principles of macromolecular drugs. CRC Crit. Rev. Ther. Drug Carrier Syst., *6:* 193–210, 1989.

13. Duncan, R., Kopeckova-Rejmanova, P., Strohalm, J., Hume, I., Cable, H. C., Pohl, J., Lloyd, J. B., and Kopecek, J. Anticancer agents coupled to [*N*-(2-hydroxypropyl)methacrylamide] copolymers. 1. Evaluation of daunomycin and puromycin conjugates *in vitro*. Br. J. Cancer, *55:* 165–174, 1987.

14. Banker, G. S. Pharmaceutical applications of controlled release: an overview of the past, present and the future. *In:* R. S. Langer, and D. L. Wise (eds.), Medical Applications of Controlled Release, Vol. 2, pp. 1–34. Boca Raton, FL: CRC Press, 1984.

15. DeDuve, C., De Barsy, T., and Poole, B. Lysosomotropic agents. Biochem. Pharmacol., *23:* 2495–2531, 1974.

16. Kartner, N., Evernden-Porelle, D., Bradley, G., and Ling, V. Detection of P-glycoprotein in multidrug resistant cell lines by monoclonal antibodies. Nature (Lond.), *316:* 820–823, 1985.

17. Ringsdorf, H. Structures and properties of pharmacologically active polymers. J. Polymer Sci. Polymer Symp., *51:* 135–153, 1975.

18. Duncan, R. Drug-polymer conjugates: potential for improved chemotherapy. Anti-Cancer Drugs, *3:* 175–210, 1992.

19. Kopecek, J., and Bazilova, H. Poly[*N*-(hydroxypropyl)-methacrylamide]—1. Radical polymerisation and copolymerisation. Eur. Polymer J., *9:* 7–14, 1973.

20. Rihova, B., and Kopecek, J. Biological properties of targetable poly[*N*-(2-hydroxypropyl)methacrylamide)-antibody conjugates. J. Controlled Release, *2:* 289–310, 1985.

21. Rihova, B., Ulbrich, J., Strohalm, J., Vetvicka, V., Bilej, M., Duncan, R., and Kopecek, J. Biocompatibility of [*N*-(2-hydroxypropyl) methacrylamide) copolymers containing Adriamycin. Immunogenicity, effect of haematopoietic stem cells in bone marrow *in vivo* and effect on mouse splenocytes and human peripheral blood lymphocytes *in vitro*. Biomaterials, *10:* 335–342, 1989.

22. Rejmanova, P., Kopecek, J., Duncan, R., and Lloyd, J. B. Stability in rat plasma and serum of lysosomally degradable oligopeptide sequences in *N*-(2-hydroxypropyl)methacrylamide) copolymers. Biomaterials, *6:* 45–48, 1985.

23. Cassidy, J., Duncan, R., Morrison, G. J., Strohalm, J., Plocova, D., Kopecek, J., and Kaye, S. B. Activity of [*N*-(2-hydroxypropyl)methacrylamide) co-polymers containing daunomycin against a rat tumour model. Biochem. Pharmacol., *38:* 875–879, 1989.

24. Seymour, L. W., Ulbrich, K., Steyger, P. S., Brereton, M., Subr, V., Strohalm, J., and Duncan, R. Tumour tropism and anti-cancer efficacy of polymer-based doxorubicin prodrugs in the treatment of subcutaneous murine B16F10 melanoma. Br. J. Cancer, *70:* 636–641, 1994.

25. Duncan, R., Cable, H. C., Lloyd, J. B., Rejmanova, P., and Kopecek, J. Degradation of side-chains of *N*-(2-hydroxypropyl)methacrylamide) copolymers by lysosomal thiolproteinases. Biosci. Rep., *2:* 1041–1046, 1982.

26. Seymour, L. W., Ulbrich, K., Strohalm, J., Kopecek, J., and Duncan, R. Pharmacokinetics of polymer-bound Adriamycin. Biochem. Pharmacol., *39:* 1125–1131, 1990.

27. Minko, T., Kopeckova, P., Pozharov, V., and Kopecek, J. HPMA copolymer bound Adriamycin overcomes MDR1 gene encoded resistance in a human ovarian carcinoma cell line. J. Controlled Release, *54:* 223–233, 1998.

28. Duncan, R., Coatsworth, J. K., and Burtles, S. Preclinical toxicology of a novel polymeric antitumour agent, HPMA copolymer-doxorubicin (PK1). Hum. Exp. Toxicol., *17:* 93–104, 1998.

29. Cassidy, J., Paul, J., Soukop, M., Habeshaw, T., Reed, N. S., Parkin, D., and Kaye, S. B. Clinical trials of nimodipine as a potential neuroprotector in ovarian cancer patients treated with cisplatin. Cancer Chemother. Pharmacol., *44:* 161–166, 1998.

30. Miller, A. B., Hoogstraten, S., Staquet, M., and Winkler, J. Reporting results of cancer treatment. Cancer (Phila.), *74:* 207–214, 1981.

31. Bolster, A. A., and Hilditch, T. E. Dosimetric considerations in ^{131}I-MIBG therapy for neuroblastoma in children. Br. J. Radiol., *68:* 481–490, 1995.

32. Fraier, D., Frigerio, E., Pianezzola, E., Strolin-Benedetti, M., Cassidy, J., and Vasey, P. A. A sensitive procedure for the quantitation of free and *N*-(2-hydroxypropyl)methacrylamide polymer-bound doxorubicin (PK1) and some of its metabolites, 13-dihydrodoxorubicin, 13-dihydrodoxorubicinone and doxorubicinone, in human plasma and urine by reversed-phase HPLC with fluorimetric detection. J. Pharm. Biomed. Anal., *13:* 625–633, 1995.

33. Beal, S. L., Sheiner, S. L., and Boeckman, A. J. NONMEM IV User's Guide, Parts I–IV. San Francisco: NONMEM Project Group, University of California, San Francisco, 1989–1994.

34. Forssen E. A., and Ross, M. E. DaunoXome treatment of solid tumours: preclinical and clinical investigations. J. Liposome Res., *4:* 481, 1994.

35. Gill, P. S., Espina, B. M., Muggia, F., Cabriales, S., Tulpule, A., Esplin, J. A., Leibman, H. A., Forssen, E., Ross, M. E., and Levine, A. M. Phase I/II clinical and pharmacokinetic evaluation of liposomal daunorubicin. J. Clin. Oncol., *13:* 996–1003, 1995.

36. Gabizon, A., Catane, R., Uziely, B., Kaufman, B., Tamar, S., Cohen, R., Martin, F., Huang, A., and Barenholz, Y. Prolongation of the circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. Cancer Res., *54:* 987–992, 1994.

37. Goebel, F-D., Goldstein, D., Goos, M., Jablonowski, H., and Stewart, J. S., for the International SL-DOX Study Group. Efficacy and safety of stealth liposomal doxorubicin in AIDS-related Kaposi's sarcoma. Br. J. Cancer, *73:* 989–994, 1996.

38. Uziely, B., Jeffers, S., Isaacson, R., Kutsch, K., Wei-Tsao, D., Yehoshua, Z., Libson, E., Muggia, F. M., and Gabizon, A. Liposomal doxorubicin: antitumour activity and unique toxicities during two complementary Phase I studies. J. Clin. Oncol., *13:* 1777–1785, 1995.

39. Duncan, R., Seymour, L. C. W., and Scarlett, L. *N*-(2-Hydroxypropyl)methacrylamide copolymers with pendant galactosamine residues. Fate after intravenous administration to rats. Biochim. Biophys. Acta, *880:* 62–71, 1986.

40. Ripamonti, M., Pezzoni, G., Pesenti, E., Pastori, A., Farao, M., Bargiotti, A., Suarato, A., Spreafico, F., and Grandi, M. *In vivo* antitumour activity of FCE-23765, a methoxymorpholino derivative of doxorubicin active on doxorubicin-resistant tumour cells. Br. J. Cancer, *65:* 703–707, 1992.

41. Vasey, P. A., Bissett, D., Strolin-Benedetti, M., Poggessi, I., Breda, N., Adams, L., Wilson, P., Pacciarini, M. A., and Cassidy, J. Phase I clinical and pharmacokinetic study of (FCE 23762). Cancer Res., *55:* 2090–2096, 1995.

42. Angelucci, F., Ballinari, D., and Farao, M. Synthesis and biological evaluation of polymer-bound 3′-deamino-3′-(2-methoxy-4-morpholinyl)doxorubicin. *In:* Proc. XIII International Symposia on Medicinal Chemistry, September 19–23, 1994. Paris.

43. Duncan, R., Seymour, L. W., O'Hare, K. B., Flanagan, P. A., Wedge, S., Hume, I. C., Ulbrich, K., Strohalm, J., Subr, V., Spreafico, F., Grandi, M., Ripamonti, M., Farao, M., and Suarato, A. Preclinical evaluation of polymer-bound doxorubicin. J. Controlled Release, *19:* 331–346, 1992.

Clinical Cancer Research

Phase I Clinical and Pharmacokinetic Study of PK1 [*N*-(2-Hydroxypropyl)methacrylamide Copolymer Doxorubicin]: First Member of a New Class of Chemotherapeutic Agents—Drug-Polymer Conjugates

Paul A. Vasey, Stan B. Kaye, Rosemary Morrison, et al.

Clin Cancer Res 1999;5:83-94.