

Phase I and Pharmacokinetic Study of PKC412, an Inhibitor of Protein Kinase C

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Purpose: *N*-Benzoyl staurosporine (PKC412) is a protein kinase C inhibitor with antitumor activity in laboratory models. We determined the toxicity of oral PKC412 administered daily for repeat cycles of 28 days.

Patients and Methods: Thirty-two patients with advanced solid cancers were treated at seven dose levels (12.5 to 300 mg daily) for a total of 68 cycles.

Results: The most frequent treatment-related toxicities were nausea, vomiting, fatigue, and diarrhea. At the two top dose levels (225 and 300 mg/d), 15 of 16 patients experienced nausea/vomiting (common toxicity criteria [CTC], version 1), grade 2 in nine of 16 and grade 3 in three of 16 patients; and six of 16 patients developed CTC grade 2 diarrhea. After 1 month of treatment, there were significant reductions in circulating lymphocyte ($P < .02$) and monocyte ($P < .01$) counts in patients receiving doses ≥ 100 mg/d. Nevertheless, only two patients developed myelosuppression (both grade 2). Of two patients with progressive cholangio-

carcinoma, one attained stable disease lasting 4.5 months and one a partial response lasting 4 months. There was a linear relationship between PKC412 dose and area under the curve_{0-24 hours} and maximum plasma concentration with marked interpatient variability. The estimated median elimination half-life was 1.6 days (range, 0.9 to 4.0 days), and a metabolite with a median half-life of 36 days was detected. Steady-state PKC412 plasma levels at the top three dose cohorts (150 to 300 mg) were five to 10 times the cellular 50% inhibitory concentration for PKC412 of 0.2 to 0.7 $\mu\text{mol/L}$.

Conclusion: PKC412 can be safely administered by chronic oral therapy, and 150 mg/d is suitable for phase II studies. The pharmacokinetics and lack of conventional toxicity indicate that pharmacodynamic measures may be additionally needed to optimize the drug dose and schedule.

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PROTEIN KINASE C family members (PKCs) are intimately involved in processes that are perturbed in cancer cells, including growth factor-mediated signal transduction, nuclear oncogene activity, cell cycle control, and topoisomerase activity.¹⁻³ Tumor promoters activate PKC, and PKC activities are elevated in a variety of tumors.⁴ High levels of PKC have been found in breast, lung, and stomach carcinomas, but low levels have been described in other tumors such as colon.⁴⁻⁷ Manipulation of various PKC isoenzymes by transfection and antisense oligonucleotide experiments indicate a role in tumorigenicity and cell differentiation that is likely to be dependent on isoenzyme expression.² Whether effects on particular PKC isoenzymes would enable selective tumor targeting is unknown. Given the multifunctional role of PKCs in tumour biology, the inhibition of PKC function would be expected to interfere with some signal transduction pathways critical for continued tumor growth and survival. We describe here our early clinical experience with the PKC inhibitor *N*-Benzoyl staurosporine (PKC412).

PKC412 is an *N*-Benzoyl derivative of the naturally occurring alkaloid staurosporine.⁸ It is an inhibitor of several kinases but predominantly the PKC enzyme family. PKC enzymes comprise more than 12 isoenzymes which can be classified into the following three main groups: (1) conventional calcium- and diacylglycerol (DAG)-dependent; (2) calcium-independent but DAG-dependent; and (3)

calcium-independent and DAG-unresponsive.⁹ The various PKC subtypes show distinct enzymologic properties, differential tissue expression, and have different modes of activation and oncogenic potential. PKC412 preferentially inhibits the conventional PKCs α , β , γ , and the calcium-independent PKCs δ , ϵ , and η .^{8,10,11} PKC412 also inhibits tyrosine kinase pathways,¹² including inhibition of vascular endothelial growth factor receptor (flk1, flt1) tyrosine kinases.¹³ These properties suggest that the drug may have antiangiogenic effects. Indeed PKC412 was recently reported to inhibit choroidal neovascularization in mice.¹⁴

PKC412 is an effective antiproliferative agent against various tumor and normal cell lines in vitro and in xenograft models.^{8,15-17} It synergizes with conventional cytotoxic agents without overt toxicity and is able to reverse p-glycoprotein-mediated multidrug resistance in vitro^{18,19}

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Table 1. Principle Toxicities of Study: Number of Patients Experiencing Toxicity per Dose Group

Total Daily Dose (mg)	No. of Patients												
	Total	Nausea			Vomiting			Diarrhea			Lethargy/Fatigue		
		Grade	1	2	3	Grade	1	2	3	Grade	1	2	3
12.5*	3	2	0	1	2	1	0	0	0	0	0	2	1
25†	3	1	0	0	1	0	0	0	0	0	3	0	0
50†	2	0	0	0	0	0	0	0	0	2	0	1	
100†	3	0	0	1	1	1	0	2	0	0	2	0	1
150‡	4	3	0	0	0	1	1	0	2	0	0	0	1
225‡	9	3	5	0	4	3	0	2	2	0	2	3	2
300‡	7	1	3	1	1	2	1	2	5	0	1	0	1

NOTE. All patients receiving 300 mg/day given noncorticosteroid antiemetic prophylaxis worst toxicities are shown. For adverse events that were not covered by NCI-CTC: Grades 1, 2, 3 = mild, moderate, and severe.

*Drug given once a day.

†Drug given twice a day.

‡Drug given three times a day.

and in vivo.²⁰ The 50% inhibitory concentration (IC₅₀) for PKC412 against malignant cells using in vitro proliferation assays was between 0.2 and 0.7 $\mu\text{mol/L}$,⁸ and this concentration may represent a minimal target for in vivo efficacy.

In dogs and mice, no toxicologic effects were observed after 3 months of treatment at doses of 1 and 10 mg/kg/d, respectively.²¹ At daily doses of 20 mg/kg/d in rats and 3 mg/kg/d in dogs, suppressive effects were observed on proliferating tissues, particularly bone marrow, testes, and intestine. Variable duration and prolongation of the ECG PQ interval was observed with high-dose therapy in dogs (30 mg/kg/d). Single-dose pharmacokinetic studies in healthy human volunteers showed maximum plasma concentrations 1 to 3 hours after dosing, with variable terminal elimination half-lives of between 5 and 31 hours.

Because of the potential anticancer activity of PKC412, we performed a phase I dose escalation study in patients with tumors refractory to conventional chemotherapy or for whom there was no conventional treatment available. PKC412 was initially given once daily and during dose escalation increased to three times daily. Patients were closely assessed for drug tolerability by both clinical and laboratory criteria.

PATIENTS AND METHODS

Patients

Patients had histologically proven cancer that was either refractory to conventional chemotherapy or unresponsive to standard treatment. Other eligibility criteria included the following: age greater than 18 years, World Health Organization performance status ≤ 2 , life expectancy of more than 3 months, and no chemotherapy or investigational agents in the previous 30 days (40 days for mitomycin C or nitrosoureas). Patients with uncontrolled heart failure, severe ischemic heart disease, and any degree of atrioventricular block were ineligible.

Eligible patients had to have a neutrophil count $\geq 2 \times 10^9/\text{L}$, hemoglobin $\geq 10 \text{ g/dL}$, platelet count $\geq 100 \times 10^9/\text{L}$, a normal serum creatinine and bilirubin, and serum transaminases \leq two times the upper limit of normal.

Study Design

PKC412 was formulated with gelucire in 12.5- and 25-mg soft gelatine capsules. Seven dose levels were evaluated. The lowest was 12.5 mg once daily, and the highest was 100 mg, tid (Table 1). The lowest dose (12.5 mg/d) was one fifth of the no adverse effect level in dogs, the most sensitive species. Toxicities were defined by the National Cancer Institute common toxicity criteria (NCI-CTC), version 1. The maximum-tolerated dose (MTD) was defined as that at which at least three out of six patients had grade 3 hematologic toxicity, two out of six patients had grade 4 hematologic toxicity, or two out of six patients had grade 3 nonhematologic toxicity. Three patients were treated at each dose level. Provided none of these patients experienced any grade II toxicities (excluding alopecia and nausea and vomiting), the dose was escalated to the next treatment level for ensuing patients. If any patient developed grade II toxicity or more (excluding alopecia and nausea and vomiting) at any dose level, a total of six patients were enrolled at that dose to further evaluate the toxicity. Escalation was planned to the MTD or to a predefined dose of 300 mg/d because 12 capsules per day was considered the maximum number feasible for compliance.

A treatment cycle comprised 28 days of daily dosing with PKC412, followed by a 7-day treatment-free period. During the first 24 hours of treatment, patients were observed in the hospital. If PKC412 was tolerated in the first treatment cycle and there was no evidence of disease progression, patients continued for a second cycle to a planned maximum of six. Because of prolongation of the ECG PQ interval observed in dogs receiving high-dose PKC412, patients were monitored for cardiac conduction changes by 24-hour continuous ECG before and during the first 24 hours of therapy.

Toxicity and Response Measurements

Patients were seen weekly during the first treatment cycle and completed daily symptom diary cards. Response to drug was assessed by a full clinical examination at the end of each cycle and by formal

staging with appropriate radiologic investigations after two cycles. For patients who continued for more than two cycles, formal staging was repeated after each ensuing two cycles.

Treatment Compliance

Compliance was defined as 80% of the total number of capsules administered. Patient compliance was checked by a capsule count at each visit.

Pharmacokinetics

Each patient took the first PKC412 dose after an overnight fast. Two hours after drug administration, patients ate a standardized light breakfast. Pharmacokinetic sampling was performed immediately before and 1, 2, 4, 6, 8, 12, and 24 hours after the first dose. Thereafter blood for pharmacokinetic analysis was obtained at weekly intervals during the first treatment cycle. Samples were collected into 5-mL heparin-coated tubes, centrifuged at 4,000 rpm for 5 minutes at room temperature in the dark within 5 minutes of collection, and the plasma transferred to brown nontransparent vials and frozen immediately at -70°C . Concentrations of PKC412 in plasma were determined at the Cancer Research Campaign Beatson Laboratories, Glasgow, United Kingdom, by a high-performance liquid chromatography method with fluorescence detection.²² The limit of quantitation was 10 nmol. The method was validated in the center and cross-checked with Novartis Pharma AG, Basel, Switzerland. Serum alpha-1 acid glycoprotein (AAG) concentrations (upper limit of normal, 20 uM) were measured at the Protein Reference Unit, Sheffield, United Kingdom, by automated immunoturbidimetric assay adapted to the Cobas Mira analyzer (Roche Diagnostics, Indianapolis, IN).²³

Ethical Considerations

The study was approved by the Central Oxford Research Ethics Committee and by the Glasgow West Ethics Committee and conducted according to the Declaration of Helsinki. Each patient provided written informed consent.

Statistics

Correlations between daily PKC412 dose and area under the curve (AUC)_{0-24 hours} and maximum plasma concentration (C_{\max}) were sought using the unweighted least squares method. Differential WBC counts were compared by Student's *t* tests.

RESULTS

Thirty-two patients entered the study. Their demographic details are listed in Table 2. A total of 68 cycles were given (median, two cycles; range, one to 12 cycles). Fifteen patients received a single cycle, 13 received two cycles, and four received more than two cycles (four, five, six, and 12 cycles). Eight patients did not complete the first treatment cycle, three because of nausea and vomiting and five because of progressive disease; although three of these five patients did receive the full 28-day treatment.

The majority of patients ($n = 22$) were withdrawn from the study because of progressive disease. Five were withdrawn because of nausea and vomiting, not controlled by standard antiemetic therapy. A further three patients with-

Table 2. Patient Characteristics

Characteristics	No. of Patients
Total	32
Age, years	
Median	62
Range	36-76
Male	16
Female	16
WHO performance status	
0	11
1	21
Site of primary tumor	
Colon	11
Adenocarcinoma unknown primary	4
Breast	3
Melanoma	2
Others	12
Previous treatment	
Chemotherapy	30
Radiotherapy	14
Neither	2

Abbreviation: WHO, World Health Organization.

draw consent. One patient with cholangiocarcinoma treated at dose level 2 (25 mg/d) attained shrinkage in his omental metastasis that fulfilled criteria for a partial response lasting 4 months. The disease had previously progressed on fluorouracil treatment. He continued on treatment for 12 cycles but was withdrawn after a cerebral vascular accident. This was attributed to a cancer associated thrombotic tendency that was manifest before starting PKC412 therapy. A second patient with cholangiocarcinoma, who had progressive disease on cisplatin-based chemotherapy before starting PKC412 300 mg/d, attained stable disease for 4.5 months.

Toxicity

The principal toxicities attributed to PKC412 were nausea/vomiting, diarrhea, and fatigue. Grade 2 toxicity occurred more frequently at dose levels of 225 and 300 mg/d (Table 1). Five patients stopped taking the drug because of nausea and vomiting, three of these before completing the first cycle of chemotherapy. At the top two dose levels, eight of 15 patients experienced grade 2 nausea, and one experienced grade 3 nausea. Five of these patients experienced grade 2 vomiting, and one experienced grade 3 vomiting. At lower doses, only two patients experienced grade 2 or more nausea (12.5 and 100 mg/d; both grade 3), and three experienced grade 2 vomiting. Once this side effect became manifest, patients received antiemetic prophylaxis with metoclopramide 10 mg four times daily, starting the evening before the first drug dose. The nausea and vomiting did not generally worsen during a cycle of chemotherapy. The diarrhea, like the nausea and vomiting,

was apparent within the first few days of treatment and dose-related. Overall, PKC412 at doses of 150 mg/d and below was generally well tolerated.

Dose-Limiting Toxicity and MTD

In this study, seven patients experienced dose-limiting toxicities. In four patients, this was grade 3 lethargy/fatigue, affecting three of 16 patients at the top two dose levels and one of three patients receiving the lowest dose. Three patients experienced grade 3 nausea/vomiting. The number of patients affected by dose-limiting toxicities did not reach the predefined criteria for MTD. However, the prevalence of symptomatic toxicities at the top two dose levels (225 and 300 mg/d) was felt to exclude these doses for chronic administration, and hence no further patients were enrolled.

Hematologic toxicity was uncommon, affecting only two heavily pretreated patients. In one patient (225 mg/d), grade 2 leukopenia occurred after 4 weeks of treatment, which did not recur after a one-level dose reduction. The second patient developed grade 2 leukopenia and grade 1 neutropenia during the second treatment cycle. The drug was stopped for 4 days, and there was no recurrence on restarting the drug at the same dose (300 mg/d). Pooling data from all patients, comparison of circulating neutrophil, lymphocyte, and monocyte counts at baseline and after 1 month of treatment showed a significant decrease only in lymphocyte counts (1.38 ± 0.54 v $1.09 \pm 0.52 \times 10^9/L$, respectively; $P < .05$). Further analysis showed that there was a dose effect of PKC412 on differential WBC counts. There were no significant changes in differential counts for patients receiving less than 100 mg/d PKC412. In patients receiving 100 mg or more per day, lymphocyte counts (1.28 ± 0.43 at baseline v $0.8 \pm 0.49 \times 10^9/L$ after 1 month; $P < .02$) and monocyte counts (0.37 ± 0.18 at baseline v $0.28 \pm 0.27 \times 10^9/L$ after 1 month, $P < .01$) were significantly lower after 1 month of treatment.

No cardiac conduction abnormalities were observed by 24-hour ECG monitoring during the first 24 hours of therapy. There were no significant effects on biochemical liver or renal function.

Compliance

Comparison of the planned dose versus the actual treatment dose, as defined by pill count, showed that a median of 96.4% of the planned dose was administered for all patients. Within each dose level, the median value was always above 80% during the treatment.

Pharmacokinetics

Pharmacokinetic data for PKC412 in the dose form used in this study had previously been determined in a single

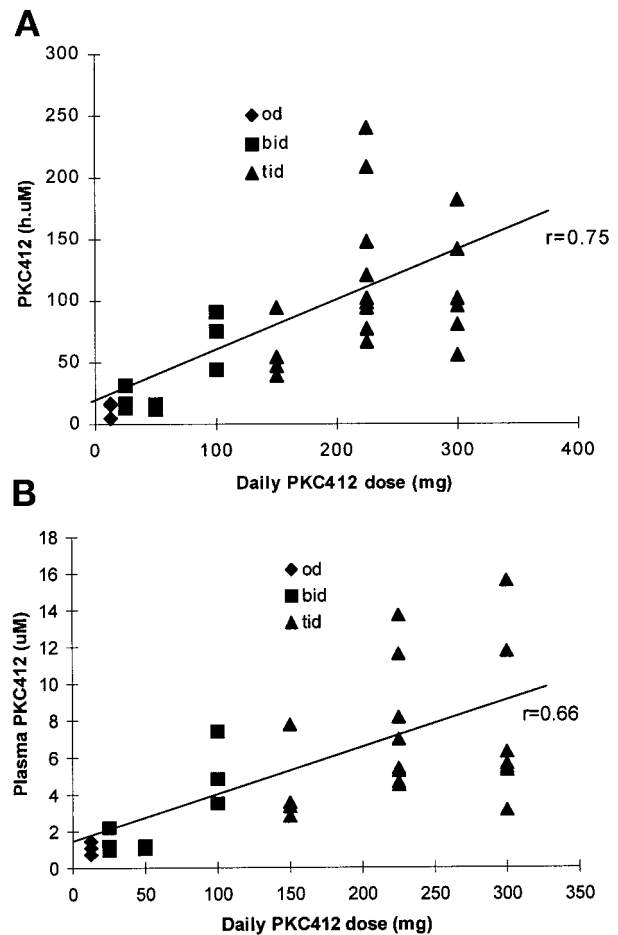


Fig 1. (A) Relationship between individual daily dose and plasma AUC (0-24 hours) values. (B) Peak drug concentration (C_{max}) and C_{max} of PKC412 in 32 patients on day 1 of repeated daily dosing of 12.5 to 300 mg given once a day, bid, or tid. Shown are the individual values and the least-squares regression line.

ascending-dose healthy human volunteer study.²¹ C_{max} of PKC412 in human volunteers were reached between 1 to 1.5 hours after dosing, and subsequent elimination of the drug from plasma was slow. The estimated terminal elimination half-lives were in the range of 5 to 29 hours.

Pharmacokinetic data for PKC412 were determined for 30 patients (Fig 1A and 1B). Mean plasma concentrations in patients at each dose level, between 1 to 24 hours after first dose on day 1, were in the range of 0.3 to 7.0 $\mu\text{mol/L}$ (Fig 2). C_{max} and $AUC_{0-24 \text{ hours}}$ increased with increasing dose, the increase being less than dose proportional. Despite the less than proportional increase with dose, there was a linear relationship between daily dose and $AUC_{0-24 \text{ hours}}$ and C_{max} (Fig 1A and 1B). There was considerable interpatient variation in PKC412 plasma levels (Fig 1A and 1B).

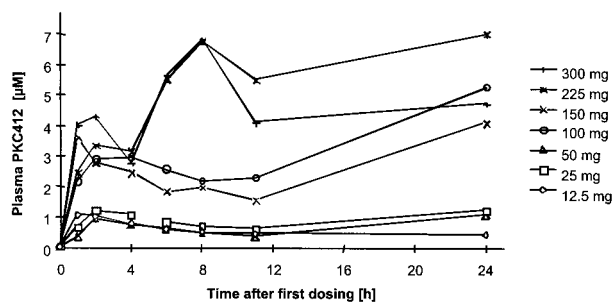


Fig 2. Mean plasma concentrations of PKC412 during first 24 hours of treatment; dose schedule: (total daily dose shown) 12.5 mg = once a day; 25, 50, and 100 mg = bid; and 150, 225, and 300 mg = tid.

PKC412 was rapidly absorbed (Fig 2), and steady-state levels for PKC412 were apparent after 1 week of daily dosing (Fig 3). The weekly drug concentrations gradually declined throughout the 4-week treatment cycle, and there were no significant differences between weekly plasma concentrations for the top three dose levels (Fig 3). In the treatment-free intervals, PKC412 concentrations decreased to near zero in most patients.

Plasma concentrations of all patients with grade 2 or greater nausea/vomiting who completed the first 28 days of treatment were analyzed in case this had affected drug compliance. Neither day 1 concentrations of PKC412 and/or metabolites nor the weekly trough levels of these patients were significantly different from the values of other patients with respect to absolute value or rate of increase or decrease in levels.

The estimated median elimination half-life of PKC412 was 1.6 days (range, 0.9 to 4.0 days; n = 12). This compared with 0.7 days (range, 0.2 to 1.3 days) in healthy

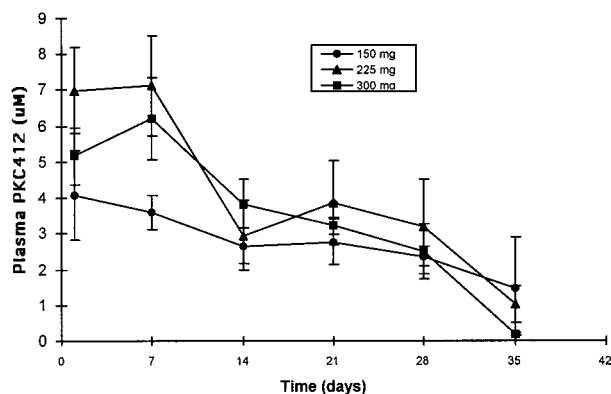


Fig 3. Weekly PKC412 plasma levels shown for dose levels 3 to 7. Patients commenced PKC412 on day 0 and stopped on day 28. Plasma measurements were performed 24 hours after commencing treatment and, thereafter, at weekly intervals. Total daily dose shown.

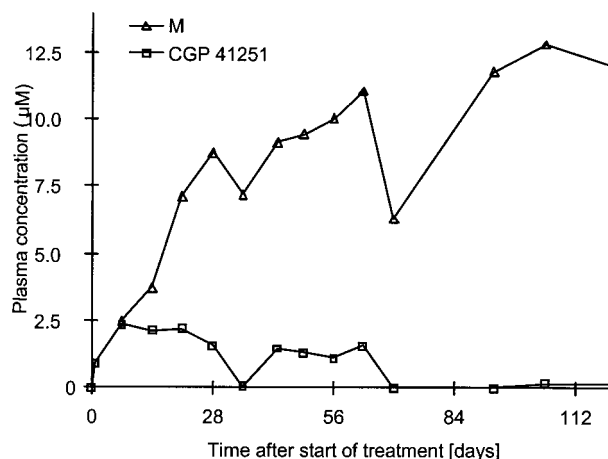


Fig 4. Typical concentration time course for PKC412A and its major metabolite M in a patient receiving treatment of 25 mg daily.

volunteers (n = 14). There was no accumulation of PKC412 (Fig 3). The plasma elimination half-life of the major PKC412 metabolite (M) was slow (median half-life of 36 days; range, 27 to 164 days). A concentration time course for PKC412 and the major metabolite in a patient receiving 25 mg daily over a 4-month period is shown in Fig 4. The biologic and chemical characteristics of PKC412 metabolites are not yet fully characterized. In vitro comparison of the effects of PKC412 and its metabolites on the activity of various PKC isoforms indicated that the metabolites had little activity compared with PKC412. Generally, the IC_{50} for the metabolites was at least five times greater than that for PKC412.

Preclinical experiments had shown an extensive binding of PKC412 to human plasma proteins, with approximately 88% to 98% protein binding, depending on the drug concentration. Binding to human (but not rodent) AAG, in particular, was significant. To understand the differences in pharmacokinetics between healthy human volunteers and study patients, plasma AAG concentrations were measured and compared with total PKC412 and metabolite concentrations. Plasma AAG levels were elevated in 21 of 24 patients in whom baseline values were obtained, and values tended to remain constant in most patients over the course of the study.

DISCUSSION

The principle toxicities in this phase I dose escalation study of PKC412 were nausea/vomiting and fatigue. Additional significant side effects were diarrhea, anorexia, and headache. At most dose levels, patients experienced some mild to moderate adverse events, but the toxicities were

more prominent at the higher dose levels. Other toxicities were uncommon, and there was no clinically important myelosuppression. There was, however, significant dose-related suppression in circulating lymphocyte and monocyte levels after 28 days of treatment in patients receiving PKC412 100 mg or more per day. Nevertheless, this myelosuppression did not manifest as significant NCI-CTC toxicity, even in patients who continued for more than one treatment cycle. This is an important observation because of the likely chronic schedule for administering this drug.

No individual toxicity reached the predefined MTD of two out of six patients with NCI-CTC grade 3 toxicity. Nevertheless, the prevalence of symptomatic toxicities at the top two dose levels (225 and 300 mg/d) clearly exclude these doses for chronic administration, whereas 150 mg/d would be an acceptable dose. The absence of a dose-limiting toxicity, as it is usually defined, contrasts with trials of conventional cytotoxic agents and indicates that it may not be appropriate to apply conventional phase I criteria to forthcoming trials of biologic response modifying agents.

In general it was apparent, within the first week of treatment, whether patients were able to tolerate the drug. Symptoms such as nausea, vomiting, and lethargy did not worsen with prolonged treatment. The nausea and vomiting could be caused by drug effects on brain tissue PKC isoenzymes. Conversely, given the diarrhea, which developed early in treatment, local effects on gastrointestinal mucosa are possible. It was not established whether dose reduction in patients who had adverse symptoms was of benefit because doses were maintained according to protocol. In the patients who continued the drug for more than one cycle, no further side effects emerged.

The pharmacokinetic data suggests that PKC412 displays less than proportional increases in plasma concentrations with increasing dose. Steady-state concentrations were reached within approximately 7 days. The decrease in weekly drug levels from 24 hours after first-dose to day 15 suggests some degree of induction of PKC412 metabolism. Thereafter, concentrations seemed to remain stable. There was considerable interpatient variation in plasma drug concentrations, particularly at the three highest dose levels, where there were no significant differences between mean weekly drug levels. Differences in gastrointestinal tract absorption and plasma protein binding may explain this variability. At the top three dose levels, plasma concentrations were consistently five to 10 times the cellular IC_{50} for PKC412 of 0.2 to 0.7 $\mu\text{mol/L}$.⁸

The plasma concentrations of PKC412 were higher and the half-life was longer than predicted from animal studies and single-dose kinetics studies in healthy volunteers.²¹ The major metabolite (M) had a particularly long half-life. Two

patients received prolonged treatment; one received 25 mg/d for 13 cycles and the other 150 mg/d for six cycles, but there was no evidence that the major metabolite accumulation produced either cumulative or delayed toxicity. The higher than predicted plasma drug levels suggests greater protein binding than anticipated. Preclinical experiments had shown extensive binding of PKC412 to human plasma proteins, with approximately 88% to 98% protein binding, depending on the drug concentration. Binding to AAG in particular was significant. It is possible that PKC412 and its metabolite preferentially bind to AAG *in vivo*. AAG levels were elevated in 21 of 24 patients tested, and this could account for the longer than anticipated plasma half-life. Recently, the staurosporine analog UCN-01 (7-hydroxystaurosporine) has been reported to exhibit strong AAG binding and to have similarly unusual pharmacokinetics, not predicted by animal studies.²⁴ Whatever the explanation, although in humans PKC412 is more protein bound than animal studies had predicted, the drug was metabolised and associated with adverse effects as well as dose-dependent pharmacodynamic changes, as discussed below.

The dynamics of dissociation from plasma proteins and tissue distribution of PKC412 are likely to be complex, and plasma levels may not be an appropriate reflection of drug concentration in target tissues. In parallel studies, we assessed whether the drug might have inhibitory effects on PKC signalling pathways *in vivo*.²⁵ This was assessed by measuring cytokine release from phytohemagglutinin-stimulated fresh whole-blood cells obtained from patients at various time points during treatment. The release of tumor necrosis factor and interleukin-6 were both significantly inhibited in a time- and dose-dependent manner. The inhibition of cytokine release at doses of 100 mg/d and above was highly consistent. At the same time points, Western blots of peripheral-blood lymphocyte lysates showed marked downregulation of extracellular signal-regulated kinase 2 (ERK2) at all PKC412 dose levels tested (50 to 300 mg/d). PKCs are involved in the regulation of these cytokines and in ERK2 phosphorylation,²⁶⁻²⁸ suggesting that PKC412 has inhibitory effects on PKCs *in vivo*.

Although this was a phase I study, tumor responses were assessed. It is, however, likely that conventional methods of assessing disease response are less valid with biologic response modifying agents than conventional cytotoxic agents. This is one of the inherent difficulties in their assessment, and other end points need to be developed. Measurement of changes in tumor metabolism by positron emission tomography using ¹⁸F-fluoro-deoxyglucose imaging is a potential suitable end point.²⁹ Biopsy of tumor to assess changes in target molecules and measurement of levels of circulating markers of tumor invasion, such as

markers of angiogenesis, are other potentially useful approaches. All these techniques, however, require validation.

Two patients with cholangiocarcinoma were treated in this study; one at a low dose (25 mg/d) and the other at a high dose (300 mg/d). The first patient attained a partial response. Before commencing PKC412, this patient had clear evidence of progressive disease both on and off conventional fluorouracil-based chemotherapy. The second patient attained stable disease, having had progressive disease with conventional cytotoxic agents. Whether PKCs are abnormally activated in cholangiocarcinomas and the role of PKCs in the etiology and progression of cholangiocarcinomas are unknown. Bile acids are potent activators of PKC,³⁰ and speculatively, this effect could have been blocked by the drug.

Signal blocking agents represent a novel approach to treating cancer. PKC412 synergizes with conventional cytotoxic agents and is able to reverse P glycoprotein-mediated multidrug resistance *in vitro*^{18,19} and *in vivo*.²⁰ In clinical practice, PKC412 could have synergistic effects when given by chronic oral administration during a course of conventional

cytotoxic chemotherapy. There was little significant hematologic toxicity, suggesting that it could be safely combined with conventional cytotoxic agents without significant overlapping toxicity. It is, however, important to note that *in vitro* data indicate that synergy between biologic response modifiers and cytotoxic agents can be schedule-dependent^{31,32} and an important consideration in combination studies.

The study suggests that PKC412 150 mg/d would be well tolerated chronically, a dose associated with plasma concentrations five to 10 times the cellular IC₅₀. It is of interest to note that two pharmacodynamic end points, suppression of mitogen-induced cytokine release and peripheral-blood mononuclear-cell ERK2 levels, and suppression of circulating lymphocytes and monocytes occurred at the same doses as significant symptomatic toxicity, suggesting similar processes could underlie these effects. Further studies examining dose and schedule in combination with conventional chemotherapeutic agents are in progress. Because plasma pharmacokinetic evaluation is complicated by protein binding and metabolism, use of biologic markers of PKC inhibition will contribute to optimizing PKC412 administration.

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