Phase I Clinical Trial of the Flavonoid Quercetin: Pharmacokinetics and Evidence for in Vivo Tyrosine Kinase Inhibition

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ABSTRACT

We have performed a Phase I clinical trial with the naturally occurring flavonoid quercetin (3,3',4',5,7-penta-hydroxyflavone). Quercetin has antiproliferative activity in vitro and is known to inhibit signal transduction targets including tyrosine kinases, protein kinase C, and phosphatidylinositol-3 kinase. Quercetin was administered by short i.v. infusion at escalating doses initially to 3-week intervals. The first dose level was 60 mg/m²; at the 10th dose level of 1700 mg/m², dose-limiting nephrotoxicity was encountered, but no myelosuppression. At the preceding dose level of 1400 mg/m², five patients were treated at 3-week intervals, and another eight patients were treated on a once-weekly schedule; overall, 2 of 10 evaluable patients had renal toxicity, 1 at grade 2 and 1 at grade 3. We therefore treated other patients at 945 mg/m² (eight at 3-week intervals and six at weekly intervals); 3 of 14 patients had clinically significant renal toxicity, 2 patients with grade 2 and 1 patient with grade 3. Patients treated on the weekly schedule did not have cumulative renal impairment but did have a fall in the glomerular filtration rate of 19 ± 8% in the 24 h after drug administration. We recommend 1400 mg/m² as the bolus dose, which may be given either in 3-week or weekly intervals, for Phase II trials.

Quercetin pharmacokinetics were described by a first-order two-compartment model with a median \( t_{1/2a} \) of 6 min and median \( t_{1/2b} \) of 43 min. The median estimated clearance was 0.28 liter/min/m², and median volume of distribution at steady state was 3.7 liter/m². In 9 of 11 patients, lymphocyte protein tyrosine phosphorylation was inhibited following administration of quercetin at 1 h, which persisted to 16 h. In one patient with ovarian cancer refractory to cisplatin, following two courses of quercetin (420 mg/m²), the CA 125 had fallen from 295 to 55 units/ml, and in another patient with hepatoma, the serum \( \alpha \)-fetoprotein fell. In conclusion, quercetin can be safely administered by i.v. bolus at a dose injection. The plasma levels achieved inhibited lymphocyte tyrosine kinase activity, and evidence of antitumor activity was seen.

INTRODUCTION

Flavonoids are found as either simple or complex glycosides in all aerial plants (1). Humans have been estimated to consume approximately 1 g flavonoids/day, the most common flavonoid glycones found in the diet being quercitrin, rutin, and robinin (2, 3). Quercetin and rutin are hydrolyzed to quercetin and robinin by the \( \beta \)-glucosidase activity of obligate anaerobes in the gastrointestinal tract (4). A recent detailed estimation of free flavonoid consumption in Western diets found the richest sources of quercetin to be onions (347 mg/kg), apples (36 mg/kg), and red wine (11 mg/kg; Ref. 5). Since the realization that many folk medicines still in use contain flavones (2), interest in this class of compounds has intensified.

Quercetin (Fig. 1) has a wide range of biological activities including inhibition of the Na\(^+\)K\(^+\) ATPase (6), protein kinase C (7), tyrosine kinases (8), HIV reverse transcriptase (with a \( K_m \) of 0.08 \( \mu M \); Ref. 9), Ca\(^{2+}\) -ATPase of sarcoplasmic reticulum (10), and pp60\( \text{src} \) kinase. Indeed, quercetin was probably the first tyrosine kinase inhibitor to be described (11), and is also reported to cause cell cycle arrest of human leukemic T cells (12) and gastric cancer cells (13) in late G(1). More recently, quercetin has been reported to induce apoptosis through a pathway involving heat shock proteins (14). Quercetin can down-regulate mutant p53 levels (15), and as mutant p53 can block apoptosis; this is another mechanism through which quercetin could facilitate cell death. Quercetin is also a potent inhibitor of phosphatidylinositol-3 kinase (16) and 1-phosphatidylinositol-4 kinase (17), important enzymes in proliferation signaling pathways (18).

Quercetin has antiproliferative activity in vitro against ovarian (19), breast (19), and stomach (20) cancer cell lines. In vivo synergy of quercetin with cisplatin against Walker lung cancer xenografts in nude mice has been described (21). Furthermore, quercetin has antiproliferative activity against human ovarian cancer primary cultures and can potentiate the action of cisplatin ex vivo (22). In primary cultures of human acute myeloid leukemia, quercetin has been demonstrated to potentiate the cytotoxic action of 1-\( \beta \)-d-arabinofuranosylcytosine (23). These activities of quercetin make it a promising candidate for Phase I evaluation in cancer patients.

Quercetin was administered in an ethanol vehicle to six normal human volunteers in 1975 at a fixed dose of 100 mg without side effects (24). We planned a dose escalation Phase I trial using 60 mg/m² as the starting dose. We have developed a novel HPLC\(^3\) method of detecting quercetin in plasma (25), and

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: HPLC, high-performance liquid chromatography; AUC, area under the curve; AFP, \( \alpha \)-fetoprotein; IC\(_{50}\), 50% inhibitory concentration; NO, nitrous oxide.
in this article describe the results of a Phase I trial of quercetin with pharmacokinetic evaluation. In addition, because quercetin is a tyrosine kinase inhibitor, we attempted to demonstrate that a pharmacodynamic effect occurred in treated patients.

**PATIENTS AND METHODS**

**Patients.** Fifty-one patients were entered into this study, the eligibility criteria was: microscopically confirmed diagnosis of cancer no longer amenable to standard therapies; age 18–75 years; performance score of ≤2 on the WHO scale; life expectancy of at least 12 weeks; informed consent; no previous chemotherapy or immunotherapy in the preceding 3 weeks or 6 weeks for nitrosoureas, mitomycin C, or extensive radiotherapy; hemoglobin >10 g/liter, WBC count >4 × 10⁹/liter, and platelet count >100 × 10⁹/liter; and reasonable serum biochemistry. All patients gave written informed consent, as approved by the Ethics Committee of the Queen Elizabeth Hospital (Birmingham, United Kingdom).

Baseline studies before administration of quercetin included: history and physical examination, blood pressure, height, weight, performance status, blood count with differential, prothrombin time, serum chemistry including bilirubin, alkaline phosphatase, aspartate aminotransferase, creatinine, urea, sodium, and potassium, urinalysis for protein and sugar, electrocardiogram, chest X-ray, and, when appropriate, tumor measurements. While on study, patients had a weekly review with clinical examination, blood count, and serum chemistry estimation. In the first part of this trial, patients were treated at 3-week intervals. Because myelosuppression was not evident with the 3-week schedule, after the dose-limiting toxicity had been encountered, weekly treatments were introduced to intensify drug delivery.

**Quercetin Administration.** Quercetin dihydrate was supplied as a pale yellow powder of >99% purity by the Aldrich Chemical Company. It was formulated under sterile conditions in a laminar flow cabinet in analytical grade DMSO (Sigma). Quercetin was reconstituted at a concentration of 50 mg/ml for doses up to 945 mg/m² and at a concentration of 100 mg/ml for higher doses. HPLC studies showed that quercetin remains stable when stored in ethanol or DMSO at 10 °C over 21 days under air at 4 °C (data not shown). Quercetin was made up in DMSO on the day of use and supplied in glass syringes. It was administered via the side arm of a polypropylene giving set through which RIMSO 50 (50% volume DMSO and 50% water) was being slowly injected. A total volume of 50 ml of RIMSO 50 was injected with each quercetin injection. This was essential to prevent precipitation of quercetin in the giving set. The rate of i.v. injection of quercetin at the starting dose of 60 mg/m² was initially rapid over 30 s, but at doses above 945 mg/m², it was increased to 5 min because of pain on injection. When the quercetin injection was completed, the drip was left in situ, and 200 ml saline were infused over the succeeding 30 min.

The starting dose of 60 mg/m² was chosen on the basis of a previous normal volunteer study (24). Thereafter, doses were increased according to a modified Fibonacci regimen (Table 2), with three patients treated at each dose level if grade 3 or 4 toxicity was not seen. The maximum tolerated dose was considered to have been reached when two of three patients or three of six patients experienced dose-limiting toxicity. Dose-limiting toxicity was defined as the production of grade 3 or 4 general toxicity, or grade 2 renal toxicity, cardiac toxicity, or neurotoxicity.

**Pharmacokinetics.** Venous blood samples for pharmacokinetics were drawn at frequent intervals, placed in Li-heparin tubes, and within 15 min separated by centrifugation. The plasma was aspirated, frozen at −70 °C, and analyzed within 2 weeks. In one patient, a sample of ascites was drawn before and 60 min after quercetin administration. Urine was collected for 24 h before and after quercetin administration and stored at −70 °C before analysis.

**HPLC Determination of Quercetin in Plasma.** Chromatographic analysis of flavonoids was performed using a Kontron HPLC system (Watford, United Kingdom) with a µ-Bondapak C₁₈ column equipped with a high-pressure mixing solvent delivery system (HPLC 422), an automatic sample injector (HPLC 465), and diode array spectrophotometric detector set to 375 nm (HPLC 440). System control, data collection, and data evaluation were performed using an IBM PC with a 450 MT₂ software data package (Kontron, Watford, United Kingdom). Standards and control samples were analyzed as previously described (25).

**Pharmacokinetic Modeling.** Quercetin plasma levels were modeled to a two-compartment open model using the Statis package based on the Marquardt algorithm. The data fitted better to a dual- than to a single-compartment model as judged by Akaika and Swartzmann's criteria. The parameters derived from the model (A, α, β, and B) allow calculation of drug clearance (ml/min), volume of distribution at steady state (liters), and AUC.

**Determination of Tyrosine Kinase Inhibition in Lymphocytes.** Twenty ml blood were taken at time intervals up to 16 h after quercetin administration. Blood specimens were immediately placed on ice and within 30 min layered onto 10 ml ice-cold Lymphoprep (GIBCO) and centrifuged for 30 min at 500 × g at 4 °C to separate the lymphocytes. The lymphocytes were aspirated, added to 40 ml ice-cold wash buffer [50 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM EGTA, 150 mM NaCl, and 100 µM sodium o-vanadate], and centrifuged at 500 × g at 4 °C for 10 min. The supernatant was removed, and the pellet was resuspended in another 20 ml ice-cold wash buffer and
recentrifuged as described above. The supernatant was then removed, and the pellet was resuspended in 500 μl lysis buffer [50 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM EGTA, 100 μM sodium o-vanadate, 100 μM phenylmethylsulfonyl fluoride, 25 mM benzamide, 50 μg/ml aprotinin, 5 μg/ml leupeptin, and 2% Triton X-100] and incubated on ice for 15 min prior to being centrifuged at 13,500 rpm for 5 min. Lymphocyte lysates were stored at −80°C.

Lymphocyte lysates from an individual patient had protein content estimated to ensure equal loading of lanes and then were subjected to SDS-PAGE (8% acrylamide gel). Proteins were transferred onto polyvinylidene difluoride membranes (Millipore) over 4 h at 95 mV in blotting buffer comprised of 25 mM Tris-HCl (pH 8.3), 192 mM glycine, and 1% SDS in 20% (v/v) methanol. After the transfer, nitrocellulose filters were washed in PBS-Tween, and blocked for 2 h at 37°C in blocking buffer containing 5% fat-free dried milk in PBS-Tween (without azide). The membranes were then incubated with the horseradish peroxidase-conjugated polyclonal antibody RC-20 (Affiniti) at 4°C overnight. After washing with PBS-Tween, the strips were incubated in detection solution containing luminol in PBS-Tween, and exposed to Hyperfilm-ECL (Amersham) for 40 mm.

**RESULTS**

**Patient Population.** Table 1 lists the characteristics of the patients treated in this study. The median age was 56 (range, 23–78) years. The tumor histologies of the treated patients were: large bowel, 14; ovary, 10; pancreas, 7; melanoma, 6; stomach, 5; renal, 2; hepatoma, 3; and non-small cell lung, 2. Two patients had other diagnoses: one with testicular teratoma and one with gastrinoma.

**Dose Escalation and Toxicity.** Table 2 shows the dose levels and number of patients treated at each dose level. Until renal toxicity was encountered, quercetin was given without prehydration. There was no hematological toxicity, neurological toxicity, mucositis, or alopecia.

**Toxicity during Bolus Injection.** At the first dose level, the only toxicity was mild local pain on injection, which lasted for the duration of injection and for perhaps 10 s afterwards. Following injection there was a brief period of flushing that affected the whole body and was associated with sweating. This lasted for approximately 3 min, but was not associated with any fall in blood pressure.

At and above quercetin doses of 945 mg/m², the concentration of quercetin injected was increased from 50 to 100 mg/ml. This was done in an attempt to minimize the volume of DMSO injected. It was immediately apparent that at this dose level there was more severe pain on injection, which was localized to the muscles in the upper arm on the side of the injection. In an attempt to minimize this, the duration of quercetin slow bolus injection was increased to 5 min, and 10 mg morphine elixir were given before drug administration. Although no formal assessment of the pain score was made, this seemed more tolerable and decreased the subjective intensity of pain and flushing.

To investigate whether bolus injection of the vehicle caused flushing and pain, two patients received 50 ml RIMSO 50 solution by rapid injection. These patients did not experience any side effects, and it was therefore concluded that quercetin and not the vehicle was responsible for pain and flushing on slow bolus injection. Quercetin administered to a patient at the 630-mg/m² dose level via a Hickman catheter resulted in no pain but slight flushing on injection.

During the dose escalation at 1400 mg/m², patients also reported dyspnea during and for a few minutes after quercetin administration. This was difficult to quantify, but the patient treated at 2000 mg/m² had severe dyspnea which lasted 5 min following quercetin administration. We considered this unacceptable and treated the next cohort of patients at 1700 mg/m², at which dyspnea was less prominent.

**Emesis.** At the 10th dose level (1700 mg/m²), grade 4 emesis was encountered. This occurred within minutes of quercetin administration, which has been the pattern in all patients with quercetin-induced vomiting. Subsequently, the 5-HT₃ antagonist granisetron (3 mg) combined with dexamethasone (8 mg) were given i.v. before quercetin. Comparing vomiting at
doses of 945 mg/m² and above, without antiemetics, 4 of 12 courses were associated with grade 2–4 vomiting versus 6 of 27 with antiemetics. Thus, the antiemetics had no significant effect (Table 3).

Cardiovascular Toxicity. One patient with a history of hypertension had renal impairment (grade 1), but her hypertension was grade 3 with a blood pressure of 230/130 mm Hg when renal impairment ensued. Her pretreatment serum creatinine was 94 μM and creatinine clearance was 69 ml/min. In the 24 h after treatment, this fell to 46 ml/min, and 1 week later her serum creatinine was 234 μM and creatinine clearance was 26 ml/min. Within 2 weeks, her creatinine clearance had normalized to 72 ml/min, and her hypertension had been controlled with atenolol.

Two other cardiovascular events occurred, both in patients treated at 945 mg/m². One patient with a previous history of angina developed central chest pain 24 h after the second course of quercetin and died on the second day after treatment. Another patient developed grade 3 nephrotoxicity after the second course of quercetin, developed back pain, and died in heart failure 16 days after the second course. A postmortem examination revealed aortic dissection extending beyond the renal arteries.

Renal Toxicity of Quercetin. Three patients were treated at 1700 mg/m², all of whom experienced renal toxicity, with elevation of serum creatinine (Table 4). These patients also experienced nausea and vomiting within minutes of the quercetin injection. In one patient (Q26) treated at 1700 mg/m², nausea was grade 4 (Table 3) and more prolonged. This may be because this patient developed grade 4 renal toxicity, with serum creatinine reaching a peak of 2145 μM 11 days after treatment, which returned to normal by day 34 (Fig. 2A). Two other patients were treated at this level: one experienced grade 2 renal toxicity and the other grade 1 toxicity, but in patient Q27 the serum creatinine remained elevated at 246 μM on day 34 posttreatment, and in patient Q28 the serum creatinine was elevated at 149 μM on day 28. None of the patients who experienced renal toxicity had evidence of nephritis, infection, or obstructive uropathy on ultrasound scanning of the abdomen.

Dose Escalation with Weekly Treatment and Nephrotoxicity. At the highest doses of quercetin administered (2000 and 1700 mg/m²), myelosuppression was not seen, but there were dose-limiting toxicities. We therefore attempted to dose intensify by using a weekly schedule. During this weekly schedule, a concerted effort was made to monitor renal function with 24-h creatinine clearances before and immediately after quercetin therapy. For 11 courses at 945 mg/m² and for 2 at 1400 mg/m², the mean pretreatment creatinine clearance was 69.5 ml/min, falling to 56.6 ml/min after quercetin treatment (P = 0.032, paired t test). Since no myelosuppression was seen with the 3-week schedule, the dose of 1400 mg/m² was explored in a more intensive weekly schedule. All of the data given are for treatments given without i.v. hydration. The first patient treated on the weekly schedule at this dose had an unresectable pancreatic cancer. Following quercetin therapy, he had prolonged nausea and developed acute renal failure (grade 4) requiring peritoneal dialysis. Another two of seven patients treated with this dose level on the weekly schedule had renal toxicity: one patient had grade 1 and one patient had grade 2.

Four patients on the weekly schedule had three or more courses of quercetin. Fig. 2B shows pretreatment creatinine clearance versus day on study. There was no cumulative fall in creatinine clearance with multiple weekly treatments, indicating that any nephrotoxicity due to quercetin was transient and reversible within 7 days.

Overall, 2 of 10 patients who were treated at 1400 mg/m² had clinically significant nephrotoxicity (1 patient with grade 4, and 1 patient with grade 2). Since this had occurred with the first course of quercetin, we therefore enrolled an additional six patients at the preceding dose level of 945 mg/m² weekly. At 945 mg/m², eight patients were treated at 3-week and 6-week intervals. Overall, combining data for both schedules, 3 of 14 patients developed clinically significant renal impairment, 2 developed grade 2, and 1 developed grade 3.

Abrogation of Renal Toxicity with i.v. Hydration. One patient treated at 630 mg/m² developed grade 3 renal toxicity, with elevation of the serum creatinine to 420 μM by day 10. By day 21 this patient’s serum creatinine had normalized (Fig. 2C). This patient was keen for additional therapy and was therefore retreated, but with prehydration of 1 liter normal saline given over 1 h, and after quercetin treatment, 0.5 liter 5% dextrose was given; there was no fall in creatinine clearance. The next course in this patient was given at 945 mg/m² with hydration, and no renal toxicity ensued. In other patients, hydration was given before bolus injection of quercetin at 945 mg/m², and the pretreatment clearance was 82.3 ± 6.7 ml/min. In the 24 h after

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**Table 3** Quercetin-induced emesis<sup>a</sup>

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>Without i.v. antiemetics</th>
<th>With i.v. granisetron and dexamethasone</th>
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</thead>
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<tr>
<td>630</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>945</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>1400</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>1700</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2000</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> The number of observations refers to courses for which data were obtained and amounts to 39 of 59, or 66%, of all potentially evaluable courses.

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**Table 4** Renal toxicity and dose level

<table>
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<tr>
<th>Dose level (mg/m²)</th>
<th>CTC nephrotoxicity (creatinine)</th>
</tr>
</thead>
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<td>1</td>
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<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
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<td>1</td>
</tr>
</tbody>
</table>

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<sup>a</sup> No renal toxicity was encountered at dose levels below 630 mg/m².

<sup>b</sup> One patient not evaluable.

<sup>c</sup> Two patients not evaluable.
quercetin treatment, the creatinine clearance was 79.5 ± 19.5 mL/min. Thus, a simple outpatient hydration schedule abrogated falls in creatinine clearance.

Reduction in Serum Potassium. For 38 of 51 patients on whom data were available, there was a statistically significant fall in serum potassium, comparing day of treatment (4.32 ± 0.07 mM) with day 8 posttreatment (3.98 ± 0.10 mM, P = 0.006). We therefore analyzed the pre- and post-quercetin urine specimens for potassium and sodium content. In 10 specimens available for analysis, the mean 24-h potassium excretion before treatment was 46.8 ± 4 mmol and after quercetin was 50.9 ± 6 mmol (P = 0.28). Values for sodium were: pretreatment, 76 ± 20 mmol/day; after, 80.9 ± 17 mmol/day (P = 0.43). Thus, no significant changes in urine potassium or sodium excretion were detected.

Pharmacokinetics. The data from 11 patients were fitted to a two-compartment model. The distribution half-life was 0.7-7.8 min, with a median of 6 min. The elimination half-life was 3.8-86 min, with a median of 43 min (Fig. 3 and Table 5). The clearance was 0.23-0.84 liter/min/m², with a median of 0.28, and the median volume of distribution was 3.7 liter/m². There was an excellent correlation of 0.89 between the dose in mg/m² and AUC. There was no obvious relationship between toxicity and kinetic parameters.

The serum levels achieved immediately after injection of quercetin were in the range of 200-400 μM at 945 mg/m², with serum levels above 1 μM being maintained up to 4 h (Fig. 3). The clearance is in the range of hepatic blood flow, and at early time points after quercetin injection, new peaks appear in the HPLC trace which may be quercetin metabolites (data not shown). In one patient, ascites fluid levels of quercetin were determined 60 min after a dosage of 945 mg/m². The level in ascitic fluid was 0.34 μM, which is 20% of that of a parallel blood specimen.

Quercetin concentration was estimated in urine from eight patients in fourteen 24-h posttreatment urine samples: seven patients were treated at 945 mg/m² and one at 1400 mg/m² for one course and then at 945 mg/m² for another four courses. The mean percentage of administered quercetin excreted unchanged in the urine was 1.97 ± 0.66% (range, 0.03-7.6%), with a range
**Phase I Clinical Trial of the Flavonoid Quercetin**


Phase I trial of quercetin plus carboplatin, manuscript in preparation.

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**Table 5: Pharmacokinetic modeling**

<table>
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<tr>
<th>Patient</th>
<th>Dose (mg/m²)</th>
<th>AUC (µg/ml/min)</th>
<th>τ_{1/2α} (min)</th>
<th>τ_{1/2β} (min)</th>
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<td>43</td>
<td>0.26</td>
<td>3.90</td>
</tr>
</tbody>
</table>

*a* Patient retreated as part of a Phase I trial of quercetin combined with carboplatin.

*b* NM, not modeled better by two-compartment kinetics; Vd_{ss}, volume of distribution at steady state.

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**Antitumor Activity.** No patients achieved conventional radiological responses according to WHO criteria; however, one patient with metastatic hepatocellular carcinoma, who was treated at the first dose level (60 mg/m²), had a sustained fall in serum AFP and alkaline phosphatase (Fig. 4A). Another patient with stage 4 metastatic ovarian cancer with extensive pelvic disease and liver metastases had completed six courses of cyclophosphamide (750 mg/m²) and cisplatin (75 mg/m²) 3 months before entry into the trial. Her response to this chemotherapy was minor, and her serum CA 125 was rising progressively, reaching a level of 290 units/ml. Following treatment with two courses of quercetin at 420 mg/m², her CA 125 fell to 55 units/ml (Fig. 4B). This patient was treated with three additional courses of quercetin at 500 mg/m² [combined with carboplatin AUC 4 (26) as part of a trial of quercetin plus carboplatin], and 6 months after going on study her CA 125 was 45 units/ml, fulfilling recently suggested criteria for marker response in this disease (27).

**In Vivo Inhibition of Lymphocyte Tyrosine Kinase Activity.** In 11 patients, lymphocyte tyrosine kinase activity was investigated before and at times up to 16 h after quercetin administration (Table 6 and Fig. 5). In 2 of 11 patients, phosphotyrosine-banding patterns were unchanged up to 60 min after quercetin administration. However, for time points ≥1 h in all evaluated samples, phosphotyrosine bands of between M_r 45,000 and 60,000 were attenuated or eliminated following quercetin treatment (Table 6). At 2 h posttreatment, inhibition of phosphotyrosine banding occurred in five of five patients. The vehicle, DMSO, caused some hemolysis, which was most pronounced in the samples obtained up to 30 min after quercetin administration (Fig. 5, A and B) but which had largely disappeared at the 1 h and subsequent time points. Despite rigorous washing procedures, the hemolysis contaminated the lymphocyte lysates, resulting in a wide phosphotyrosine band of molecular weight approximately M_r 20,000, which was clearly discernible from the bands attenuated by quercetin. Attenuation of phosphotyrosine bands occurred at all dose levels (Fig. 5, C-E), and the number of bands inhibited increased with time. In one patient, inhibition of phosphotyrosine banding was apparent up to 16 h after treatment (Fig. 5G).

**DISCUSSION**

The aberrant expression of protein tyrosine kinases activated by peptide growth factors can override growth regulatory control and lead to cancer (28, 29). Nonreceptor tyrosine kinases, such as pp60^c-src, have SH2 domains which direct binding
to phosphotyrosines of receptor tyrosine kinases, further amplifying and diversifying the growth signals. Thus, it is not surprising that the development of tyrosine kinase inhibitors, tyrophostins, is an attractive concept and a very active area (30). The first tyrophostin discovered was the flavonoid quercetin, which was found to inhibit pp60 src (11). Perhaps, it is therefore appropriate that quercetin should be the first tyrophostin to be systematically tested in a Phase I cancer trial.

Quercetin was administered to six normal volunteers in 1975; at that time it was considered a drug with potentially beneficial vascular effects (24). This previously published work provided us with a safe starting dose in the absence of conventional preclinical animal toxicity.

The limit of solubility for quercetin in 500 ml 10% ethanol is 100 mg, and severe alcohol intoxication would result if higher doses were used. For this reason, we pursued alternative formulations which would allow dose escalation. The solubility of quercetin in DMSO at room temperature is 150 mg/ml. These questions then arise, how much DMSO can be administered to humans safely, and what are the potential complications? DMSO is reported to be used i.v. to lower raised intracranial pressure before emergency neurosurgical procedures (31). The doses used were initially 1 g/kg infused as a 20% solution, but doses as high as 8 g/kg/day could be given safely. DMSO is recognized as causing mild hemolysis, and the higher the concentration of DMSO the more hemolysis is observed (32). However, administration of a 40% solution of DMSO at a dose of 1 g/kg i.v. over 20 min is reported to be well tolerated (32). At a quercetin dose of 1400 mg/m², approximately 0.5 g/kg, of DMSO is given as the vehicle, which is one half of the amount previously reported as well tolerated.

On administration of quercetin, the patients noted pain, principally in the biceps muscle on the side of injection. This lasted throughout the injection and for a few minutes afterward. This pain was more severe at higher doses of quercetin and was not due to the vehicle. The speed of onset makes ischemic pain perhaps unlikely, and the direct stimulation of nociceptive fibers may be a possibility. The pain was significantly reduced by prolonging the infusion time and using 10 mg morphine elixir.

During the injection of quercetin, all of the patients experienced transient flushing for up to 5 min. This was not associated with a fall in blood pressure, and must therefore be related to dilution of blood vessels smaller than resistance vessels. Quercetin is known to have vasodilator effects, inhibiting the spontaneous myogenic contractions of rat portal vein with an IC₅₀ of 9 μM and reversing phorbol 12-myristate 13-acetate-induced aortic contractions with an IC₅₀ of 11 μM (33), suggesting involvement of protein kinase C. Other biological activities of quercetin may have contributed to the vasodilator effect, including inhibition of phosphodiesterases (34).

No clinically significant toxicities were seen until the 10 h dose level (1700 mg/m²) when renal toxicity, manifesting as elevation of serum creatinine, occurred. In one patient, this was grade 4 reaching 2145 μM, but without dialysis, serum creatinine normalized within 34 days. The mechanism of renal toxicity has not yet been elucidated, possibilities include alteration of renal blood flow or direct renal tubular damage. It is recognized that differentiating between these mechanisms is difficult, requiring invasive techniques (35). One potential biochemical mechanism is impairment of NO synthase which generates the vasodilator NO. Quercetin inhibits NO synthase with an IC₅₀ of 220 μM (36). Even small reductions in the blood flow to the renal medulla, which is in a state of incipient hypoxia due to its high metabolic rate, can lead to ischemic damage, and, if this persists, lead to acute tubular necrosis (37). Quercetin-induced renal impairment was investigated intensively in patients treated on the weekly schedule. In these patients, 24-h creatinine clearance was estimated before and after quercetin administration. Quercetin caused a 20% reduction in creatinine clearance, which recovered within 7 days.

When we encountered dose-limiting grade 4 renal toxicity at 1700 mg/m², we treated a total of five patients at 1400 mg/m² on the 3-week schedule, no myelosuppression was seen. We then attempted to increase dose intensity by giving 1400 mg/m²...
Fig. 5  *In vivo* tyrosine kinase inhibition with quercetin. Western blot analysis of pre- and postquercetin peripheral blood leukocytes using antiphosphotyrosine monoclonal antibody RC 20. A, DMSO caused hemolysis of patient samples, resulting in a wide band of low molecular weight, 26 μg lysate protein/lane. B, DMSO-induced hemolysis was rapidly cleared, a reduction being apparent at 20 min after quercetin, 35 μg lysate protein/lane. C–E, 1 h after treatment, hemolysis was no longer apparent, with 45, 23, and 62 μg lysate protein/lane, respectively. Quercetin attenuated phosphotyrosine bands of M, 45,000–60,000. The doses of quercetin and times after drug administration are indicated. F and G, 48 and 39 μg lysate protein/lane, respectively. Time courses from two patients indicate that quercetin attenuation of phosphotyrosine bands persisted for up to 16 h after treatment.
on a weekly schedule, and encountered grade 4 toxicity in the first patient treated. In the next seven patients treated at this dose level, one patient had grade 2 renal toxicity, which was considered sufficiently serious to be regarded as dose limiting. We therefore attempted to dose intensify using the previous dose level of 945 mg/m² weekly and encountered significant nephrotoxicity in one of six patients, which we felt represented an acceptable level of toxicity.

Additional patients were treated at 945 mg/m² on a 3-week schedule as part of a parallel trial to combine carboplatin and quercetin, and surprisingly encountered grade 3 renal toxicity. We therefore retreated the next patient at 630 mg/m², and this patient had reversible grade 3 toxicity. At this stage we were concerned that the nephrotoxicity might be unpredictable. This patient however normalized renal function by day 14 and was keen to continue therapy. She received prehydration with 1 liter normal saline followed by posthydration with 0.5 liters 5% dextrose. This prevented renal toxicity in this patient, and in an additional six courses of quercetin given with hydration at 945 mg/m² to five patients, no falls in creatinine clearance were seen.

Conventionally Phase I trials should lead to a recommendation of the dose to be explored in Phase II trials. Nephrotoxicity seen at 1700 mg/m² was dose limiting, but we also saw grade 3 nephrotoxicity in one patient treated at 630 mg/m². Subsequently, as other patients were studied, it became clear that simple i.v. prehydration could at least partially abrogate the nephrotoxicity of quercetin. With this in mind, the emesis and the pain on injection, we believe that 1400 mg/m² given weekly or every 3 weeks can be recommended for Phase II trials.

In the literature, quercetin has been demonstrated to have antiproliferative effects against multidrug resistant MCF-7 human breast cancer cells with a 50% effective dose of 10 μM (38), against ovarian cancer cells with an IC₅₀ of 5 μM (22), to synergize with cisplatin in a mouse xenograft model at a dose of 10 mg/kg (21), and to potentiate the actions of l-β-D-arabinofuranosylcytosine to kill human AML cells at 1 μM (23). At doses above 630 mg/m², quercetin plasma levels were in excess of 1 μM for at least 3 h.

In this Phase I trial, one patient with cisplatin refractory ovarian cancer had a large and sustained fall in serum CA 125 levels. Criteria have been proposed for the use of CA 125 as a surrogate marker of response (27), which our patient fulfills. Another patient with hepato-cellular carcinoma had a fall of α-fetoprotein from 460 to 40. Again, this fall was sustained, but not associated with radiological regression of measurable disease to be counted as a standard partial response.

If one takes a mechanistic approach to cancer drug development, then it is essential to attempt to demonstrate pharmacodynamic effects and correlate these with the drug’s pharmacokinetics. Ideally, this would involve biopsy of cancers before and after drug administration. However, human tumors can be hazardous to biopsy, and the ethics and discomfort make this approach untenable. The other option open is to use patient lymphocytes as surrogate indicators. This has been applied successfully in other areas of signal transduction research, e.g., down-regulation of G protein-coupled adrenoceptors. In blood leukocytes, measurement of O⁺-alkylguanine-DNA-alkyltransferase activity inversely correlated with the probability of response in melanoma patients treated with dimethylphenyltriazine CB 10–227 (39), and in ovarian cancer treated with cisplatin, the formation of platinum-DNA adducts in blood lymphocytes strongly predicts for response (40).

We were able to demonstrate that before quercetin administration the majority of patients had detectable phosphotyrosine bands, particularly in the molecular weight range Mₚ 40,000–60,000, and that quercetin treatment abrogated some of these bands. This approach is open to criticism. First, the tyrosine kinases of lymphocytes may be different than those in tumors. Second, we cannot be certain that tyrosine kinase inhibition is the mechanism through which quercetin exerts its antiproliferative effects. For these reasons, we have not pursued the characterization of the identified phosphotyrosine bands.

This is the first reported Phase I clinical trial with a novel class of antitumor agents, tyrphostins, in humans. We are currently refining the methodology to allow specific targets of tyrosine kinase inhibition to be identified and investigated in solid tumors and leukemic cells in ongoing clinical trials.

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