Phase II Trial of Curcumin in Patients with Advanced Pancreatic Cancer

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Abstract

Purpose: Pancreatic cancer is almost always lethal, and the only U.S. Food and Drug Administration – approved therapies for it, gemcitabine and erlotinib, produce objective responses in <10% of patients. We evaluated the clinical biological effects of curcumin (diferuloylmethane), a plant-derived dietary ingredient with potent nuclear factor-κB (NF-κB) and tumor inhibitory properties, against advanced pancreatic cancer.

Experimental Design: Patients received 8 g curcumin by mouth daily until disease progression, with restaging every 2 months. Serum cytokine levels for interleukin (IL)-6, IL-8, IL-10, and IL-1 receptor antagonists and peripheral blood mononuclear cell expression of NF-κB and cyclooxygenase-2 were monitored.

Results: Twenty-five patients were enrolled, with 21 evaluable for response. Circulating curcumin was detectable as drug in glucuronide and sulfate conjugate forms, albeit at low steady-state levels, suggesting poor oral bioavailability. Two patients showed clinical biological activity. One had ongoing stable disease for >18 months; interestingly, one additional patient had a brief, but marked, tumor regression (73%) accompanied by significant increases (4- to 35-fold) in serum cytokine levels (IL-6, IL-8, IL-10, and IL-1 receptor antagonists). No toxicities were observed. Curcumin down-regulated expression of NF-κB, cyclooxygenase-2, and phosphorylated signal transducer and activator of transcription 3 in peripheral blood mononuclear cells from patients (most of whom had baseline levels considerably higher than those found in healthy volunteers). Whereas there was considerable interpatient variation in plasma curcumin levels, drug levels peaked at 22 to 41 ng/mL and remained relatively constant over the first 4 weeks.

Conclusions: Oral curcumin is well tolerated and, despite its limited absorption, has biological activity in some patients with pancreatic cancer.

Pancreatic adenocarcinoma is one of the most lethal cancers, with most patients dying of their disease within 1 year (1). The only currently available U.S. Food and Drug Administration – approved treatments for this disease are gemcitabine and erlotinib, both of which produce responses only in a minority of patients, and their effect on survival is measured in weeks only (2, 3). Therefore, better therapies are urgently needed.

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Numerous studies have indicated that the inflammatory transcription factor nuclear factor-κB (NF-κB) is constitutively active in patients with pancreatic cancer (4). The role of NF-κB in suppression of apoptosis, tumor growth, invasion, angiogenesis, and metastasis, via a variety of downstream effectors, is well documented (5–8). Therefore, an agent that can target NF-κB is of interest for the treatment of pancreatic cancer.

Curcumin (diferuloylmethane) is derived from turmeric (*Curcuma longa*). We and others have shown that it suppresses NF-κB activation (9) as well as a multitude of other biological signals pertinent to cancer (5, 10, 11). Recent work in our laboratory showed that treatment of human pancreatic cancer cells with curcumin leads to down-regulation of constitutive NF-κB activation, suppression of NF-κB-regulated gene products, and inhibition of cell growth associated with apoptosis (12). In addition, administration of liposome-encapsulated curcumin systemically suppresses the growth of human pancreatic cancer xenografts in a mouse model, and this antitumor activity is accompanied by an antiangiogenic effect (13).

Phase I studies of curcumin have shown that this agent can be administered safely at oral doses of up to 8 g/d (14, 15). There was no dose-limiting toxicity; dosing was limited by the number of pills that patients could or would swallow daily. However, the usefulness of curcumin may be attenuated because of its poor oral bioavailability (16). Therefore, we

did the present phase II study to determine whether oral curcumin has biological activity in patients with pancreatic cancer.

Patients and Methods

Eligibility. Patients were eligible if they were at least ≥18 years old and had histologically confirmed adenocarcinoma of the pancreas. Patients had to have a Karnofsky performance status of >60 at study entry (17) and preserved hepatic function (bilirubin ≤ 2.0 mg/dL) and renal function (creatinine ≤ 2.0 mg/dL). Patients with an unstable medical condition or intercurrent illness, including uncontrolled diabetes mellitus or hypertension, active infections requiring treatment with systemic antibiotics, unstable congestive heart failure, uncontrolled arrhythmias, unstable coagulation disorders, brain metastases, and uncontrolled seizure disorder, were excluded. In addition, pregnant women and those who were breastfeeding were excluded as were individuals who underwent radiotherapy or chemotherapy <4 weeks beforehand. All subjects gave their written informed consent in keeping with the policies of the Surveillance Committee of The University of Texas M. D. Anderson Cancer Center.

Curcumin. Curcumin was obtained as a generous donation from Sabinsa in 1 g caplet form. Each capsule contained 1 g curcuminoids (900 mg curcumin, 80 mg desmethoxycurcumin, and 20 mg bisdesmethoxycurcumin) confirmed by high-performance liquid chromatography tandem mass spectrometry.

Study design. This was a nonrandomized, open-label, phase II trial of curcumin, and we are reporting on the first 25 patients who satisfied all inclusion and exclusion criteria. Patients took oral curcumin daily for 8 weeks. The starting dose was 8 g/d. The patients could not receive any concomitant chemotherapy or radiotherapy, although they could receive supportive care. Patients who had stable disease or better after 8 weeks received continued therapy with curcumin at the same dose and schedule.

Evaluation during study. A complete history [pathologic confirmation of malignancy, disease staging, prior therapy/surgery, and prior response(s)] and a physical examination, as well as blood tests (including a complete blood count with differential, platelet counts, and electrolytes), renal and hepatic function tests, a serum pregnancy test for female patients of childbearing potential, tumor markers (CA 19-9, CA 27.29, and CA125 as well as carcinoembryonic antigen), an electrocardiogram, a chest radiograph, and diagnostic imaging, were done at baseline. All of these procedures were repeated every ∼4 weeks and at the end of therapy for all patients enrolled on the study, except for diagnostic imaging, which was repeated every ∼8 weeks during the course of therapy.

Safety evaluation. Systemic and local adverse events were assessed using the National Cancer Institute expanded Common Toxicity Criteria version 3.0 (18). Patients could continue treatment until disease progression unless grade 3 toxicity supervened.

Tumor response. Tumor response was defined as a complete response, partial response, stable disease, or progressive disease as per the classic Response Evaluation Criteria in Solid Tumors criteria (19). In addition, tumor markers were evaluated at the beginning of and every ∼8 weeks during treatment.

Correlative studies. Correlative studies were done on blood samples of patients if they gave informed consent for optional blood draws for research purposes. Correlative studies done include serum cytokine assessment; the effect of orally administered curcumin on constitutive and tumor necrosis factor-α–induced binding expression of NF-κB, cyclooxygenase-2 (COX-2), and phosphorylated signal transducer and activator of transcription 3 (pSTAT3) in peripheral blood mononuclear cells (PBMC) pretherapy and on day 8 by using electrophoretic mobility shift assay (EMSA); and pharmacokinetics of curcumin.

Cytokines. Cytokines [interleukin (IL)-6, IL-8, and IL-10 and IL-1 receptor antagonists (IL-1RA)] have been implicated previously in the pathophysiology of pancreatic cancer (20). Serum samples were drawn by a phlebotomist prestudy at 24 h, 8 days, 4 weeks, and 8 weeks to assess these cytokine levels, measured using an ELISA with commercially available kits (Quantikine; R&D Systems). The lower limits of the assay sensitivity are as follows: IL-6 (0.7 pg/mL), IL-8 (3.5 pg/mL), IL-10 (3.9 pg/mL), and IL-1RA (22.0 pg/mL), respectively.

As controls, cytokine levels were measured in 48 to 62 healthy volunteers depending on the cytokine assessed. Volunteers provided informed consent in accordance with institutional policy. The control samples were frozen and stored in a manner identical to the handling of patient samples.

All serum samples were aliquoted and stored at -80°C. Baseline samples were obtained within 48 h before starting therapy. Samples were thawed and assayed in duplicate with all values expressed as the mean of the two determinations. A standard curve was generated using known concentrations of recombinant cytokines according to the manufacturer's instructions and the samples were read using a plate reader (Molecular Devices). Results were calculated by generating a four-variable, logistic curve fit using the SOFTmax Pro software program (version 2.6; Molecular Devices). The concentration of a particular cytokine was then determined using the standard curve.

Materials. The mouse monoclonal antibody (sc-8059) against pSTAT3, which detects STAT3 phosphorylated at tyrosine residue 705, and antibody against the epitope corresponding to amino acids mapping within the amino-terminal domain of human NF-κB p65 (anti-p65) were obtained from Santa Cruz Biotechnology. Anti-COX-2 antibody was purchased from Transduction Labs (now Invitrogen). The liquid 3,3′-diaminobenzidine substrate chromogen system-horseradish peroxidase used for immunocytochemistry was obtained from Dako-Cytomation. Bacteria-derived human tumor necrosis factor, purified to homogeneity with a specific activity of 5×10^7 units/mg, was provided by Genentech. Penicillin, streptomycin, RPMI 1640 and fetal bovine serum were obtained from Invitrogen. Tris, glycine, sodium chloride, SDS, and bovine serum albumin were obtained from Sigma.

NF-κB activation. To determine NF-κB activation status, we isolated the nuclei from PBMC derived from patients with pancreatic cancer and healthy volunteers, homogenates were prepared, and EMSA was carried out essentially as described previously (21). Briefly, nuclear extracts prepared from PBMC (1×10^6/mL) were incubated with ^{32}P-end-labeled 45-mer double-stranded NF-κB oligonucleotides (4 g protein with 16 fmol DNA) from the HIV long terminal repeat (5′-TTGTTACAAG*GGGACTTTC*CGCTG*GGGGACTTTC*CAGGGAGGCGTGG-3′; italics indicates NF-κB binding sites) for 15 min at 37°C. The DNA-protein complex formed was separated from free oligonucleotides on 6.6% native polyacrylamide gels. A double-stranded mutated oligonucleotide (5′-TTGTTACAACTCACTTTCCGCTGCTCACTTTCCAGG-GAGGCGTGG-3′) was used to examine the specificity of binding of NF-κB to the DNA. The specificity of binding was also examined by competition with an unlabeled oligonucleotide. For supershift assays, nuclear extracts were incubated with antibody against the p65 subunit of NF-κB for 30 min at room temperature before the complex was analyzed using EMSA. Antibodies against cyclin D1 and preimmune serum were included as negative controls. The gels were dried and visualized, and radioactive bands were quantitated using a Phosphor-Imager (Molecular Dynamics) with the ImageQuant software program.

Immunolocalization of NF-κB p65, pSTAT3, and COX-2. The nuclear localization of p65, pSTAT3, and COX-2 was examined using an immunocytochemical method as described previously (22). Briefly, PBMC derived from patients with pancreatic cancer were plated on glass slides, allowed to adhere overnight, and fixed with paraformaldehyde. After a brief washing with PBS, slides were blocked with a protein block solution (DakoCytomation) for 20 min and then incubated with rabbit polyclonal anti-human p65 antibody, mouse monoclonal anti-human pSTAT3, and anti-COX-2 antibodies (at dilutions of 1:100, 1:50, and 1:75, respectively). After incubation overnight, the slides were washed and then incubated with biotinylated link universal antiserum and then

a horseradish peroxidase-streptavidin conjugate using a labeled streptavidin-biotin system kit. Slides were rinsed and developed using 3,3′-diaminobenzidine as a chromogen. Finally, slides were rinsed in distilled water, counterstained with Mayer's hematoxylin, and mounted for evaluation using digital picture exchange. Photographs of the stained PBMC were captured using the Photometrics Coolsnap CF color camera (Nikon) and the MetaMorph software program (version 4.6.5 software; Universal Imaging).

Curcumin pharmacology

Analysis of clinical curcumin product. Curcumin used for cancer prevention is seldom administered in a pure chemical form. Rather, it typically consists of three separate curcuminoids consisting of curcumin itself as well as demethoxycurcumin and bisdesmethoxycurcumin (23, 24). To determine the qualitative and quantitative presence of these curcuminoids in the curcumin product used for this clinical trial, the drug material was separated on a Gemini 5 μm C18 (2 × 100 mm) analytic column (Phenomenex) using a linear acetonitrile/methanol/ 0.2% formic acid gradient. The amount of curcuminoid was detected using a Waters Quattro Ultima tandem mass spectrometer equipped with electrospray-positive ionization capability. All three compounds were quantified by using standard calibration curves prepared from reference standard materials obtained from Sigma-Aldrich. Calibration curves were prepared by making a 1 mg/mL stock solution of the authentic materials in methanol and then serially diluting the stock solutions to 1,000, 500, 100, 50, 10, 5, and 1 ng/mL in 50:50 methanol/0.2% formic acid. Calibration curves were then prepared using the mass spectrometry quantification software program. The percentages of the three curcuminoids in the curcumin product used in this study were as follows: curcumin, 87.2% (detected at m/z 367); demethoxycurcumin, 10.5% (detected at m/z 337); and bisdesmethoxycurcumin, 2.3% (m/z 307).

Analysis of curcumin pharmacology by mass spectrometry. Despite the use of doses of curcumin as high as 8 g/d, very little free curcumin is typically found in patient plasma samples (14, 16). Rather, curcumin is present in plasma in conjugated (glucuronide and sulfate) forms, thereby necessitating appropriate enzymatic hydrolysis of the plasma before detection of free curcumin (24). In the present study, plasma samples were obtained from patients before they received their initial dose of curcumin as well as at 1, 2, 6, 24, 48, and 72 h, day 8, and 4 weeks after day 1 while still receiving the same daily dose of

curcumin. Aliquots (200 L) of plasma were mixed with 600 L Dulbecco's PBS (Sigma-Aldrich) and 200 L of 100 units/L type II β-glucuronidase/sulfatase (total 20,000 units) in Dulbecco's PBS and incubated at 37°C. After 1 h, the incubated plasma was mixed with 1 mL of 0.2% formic acid to acidify the solution and samples were then extracted three times with 3 mL ethyl acetate. Ethyl acetate extracts were combined and evaporated to dryness with nitrogen gas; dried samples were then reconstituted with 200 L of 50:50 methanol/0.2% formic acid and analyzed for curcumin content using high-performance liquid chromatography-tandem mass spectrometry. The instrument used was an Agilent 1100 binary high-performance liquid chromatography system with a temperature-controlled autosampler connected to a Waters Quattro Ultima tandem mass spectrometer. Extracted curcumin was chromatographed using a linear gradient consisting of solution A (0.2% formic acid) and solution B (80:20 acetonitrile/methanol). The initial mobile phase conditions consisting of 30% solution A and 70% solution B were switched to 5% solution A and 95% solution B at 3 min. These conditions were maintained for 2 min before switching back to the initial conditions, which were maintained for an additional 3 min before additional sample analyses. The analytical column, Gemini 5 μm C18 (150 × 2 mm), was obtained from Phenomenex. The mass spectrometer was run in the electrospray-positive mode with curcumin being detected and quantified using the following mass transitions m/z = 369.2 > 285.0 mass transition. Quantification was done using a standard curve constructed from extracted human plasma spiked with known amounts of Sigma curcuminoid standards. The extracted curves were prepared using spiked plasma standards ranging in concentration from 1 to 1,000 ng/mL. These standard curves typically had correlation coefficients of >0.98.

Results

Patient characteristics. Of the 25 patients enrolled, 24 patients were evaluable for toxicity and 21 were evaluable for response (Table 1). Their median age was 65 years (range, 43-77 years). Thirteen patients were men. The median number of prior therapies was 2 (range, 0-4) and the median time from diagnosis to enrollment into the trial was 8 months (range, 1-67 months).

Toxicity and response. We observed no treatment-related toxic effects. To date, one patient remains stable for >18 months and another patient had a dramatic but brief tumor response. The former patient had previously undergone a failed Whipple's surgery followed by gemcitabine and radiation for locally advanced disease. He had an elevated CA125 level but not an elevated CA19.9 level. With treatment (curcumin 8 g by mouth daily), the CA125 level in this patient has slowly decreased over 1 year (Fig. 1). His weight remains unchanged (and he does not have ascites or edema). His lesions are stable in size by serial positron emission tomography/computed tomography scans and there has been a decrease in the standardized uptake value in those lesions from a baseline level of 10.6 to a level of 5.7 after 12 months of therapy. One patient had a brief but marked response (73% reduction in tumor size by Response Evaluation Criteria in Solid Tumors) that lasted 1 month (Fig. 2). Interestingly, at the time of progression, the lesions that had regressed remained small, but other lesions grew larger (data not shown). Finally, one patient remained on study for ∼ 8 months with stable weight and a feeling of well-being, albeit with progression in nontarget lesions.

Cytokine levels in healthy volunteers. The majority of healthy volunteers (n = 48-62 participants depending on the cytokine being measured) had undetectable serum levels of IL-6, IL-8,

Table 1. Patient characteristics

Characteristic	*n*
Total no. patients treated	25
No. patients evaluable for toxicity	24
No. patients inevaluable for toxicity	1*
No. patients evaluable for response	21
No. patients inevaluable for response	4 †
Median (range) age, y	65 (43-77)
Sex (men/women)	13/12
Median (range) no. prior therapies	2 (0-4)
Type of prior therapy	
Surgery	12
Radiotherapy	10
Gemcitabine alone	9
Gemcitabine with other chemotherapy	9
Erlotinib-based	1
Other chemotherapy	4
No prior therapy	3
Median (range) time from diagnosis, mo	8 (1-67)

*Gastric outlet obstruction on day 1.
† Gastric outlet obstruction on day 1 (n = 1), noncompliant (n = 1), and lost to or inadequate follow-up (n = 2).
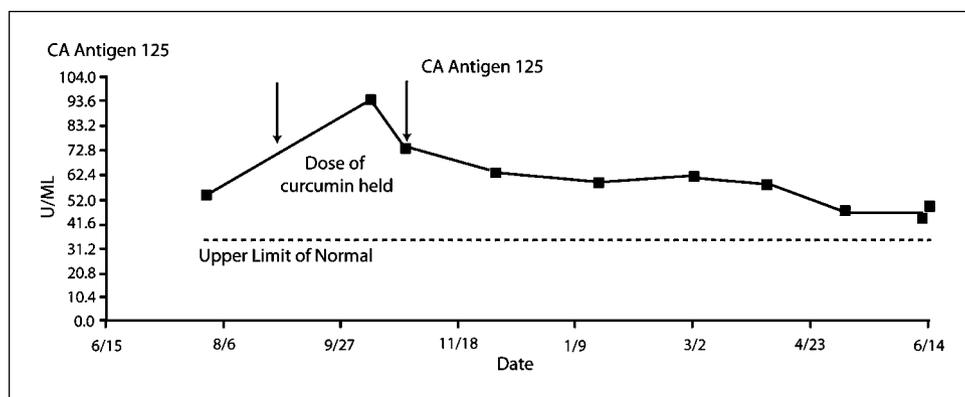
Fig. 1. Levels of CA125 in patient 14. Serum levels of CA125 increased transiently before dropping again when the dose of curcumin was held briefly (for 3 wk) for surgery for correction of an abdominal wall defect associated with his previous surgery and radiation therapy. CA125 then dropped when curcumin was restarted. CA 19-9 was not elevated.

and IL-10. In contrast, they all had detectable serum levels of IL-1RA (20).

Cytokine, NF-κB, and COX-2 levels in patients who received curcumin. As per our previous studies, baseline serum cytokines were measurable and elevated in most of the patients with pancreatic cancer (Table 2; ref. 20), including the two patients who appeared to have biological activity of curcumin after treatment. Notably, the levels were below the median for both patients for IL-6 and above the median for both patients for IL-1RA. The patient who benefited most (≥18 months with slowly improving disease) had the highest IL-1RA level among all patients.

After treatment, we detected variable changes in cytokine levels (Fig. 3A-D). Of interest, the patient who had a marked, albeit short-lived, tumor response (patient 8) had significant increases in all cytokine levels. These increases were greater than those seen in any of the other patients. Specifically, the IL-6 level reached 35-fold of the baseline level for this patient. The patient who had stable disease for ≥18 months (patient 14) experienced slow improvement over 1.5 years and had decreases in all cytokine levels. NF-κB is constitutively active in patients with pancreatic cancer.

Because NF-κB has been shown to play a critical role in the growth and angiogenesis of pancreatic cancer (25, 26), we examined the expression of this transcription factor in PBMC by immunocytochemistry (22) and by EMSA. Immunocyto-

chemistry showed constitutively active NF-κB as indicated by nuclear localization of p65 (Fig. 4A). In contrast, neither EMSA nor immunocytochemistry showed NF-κB activation in healthy volunteers (Fig. 4; representative data shown). NF-κB was constitutively active in PBMC derived from patients with pancreatic cancer as examined using a DNA-binding assay (Fig. 4B). NF-κB binding was comparable with that after tumor necrosis factor stimulation. Of the 25 patients, 19 consented to optional blood draws to look for correlative markers and cytokines. All 19 patients examined had constitutively active NF-κB (see Table 3). Ten of the 19 patients also donated their blood samples for research blood draw for immunocytochemistry at day 8 of therapy (Table 3). On treatment of patients with curcumin, immunocytochemistry showed a decline (without reaching statistical significance; $P = 0.1$, Student's t test) in nuclear NF-κB compared with that in normal volunteers (Table 3; Fig. 4C), but EMSA did not (data not shown).

We also examined COX-2 expression in PBMC because COX-2 is activated by NF-κB, is overexpressed in many tumors, including pancreatic cancer, and plays a role in tumorigenesis (6–8). PBMC in all 19 patients examined expressed COX-2 by immunocytochemistry (Table 3; Fig. 4). The COX-2 expression levels declined post-treatment with curcumin ($P < 0.03$, Student's t test).

Additionally, we examined patient blood samples for expression of activated pSTAT3 in PBMC, because pSTAT3 is
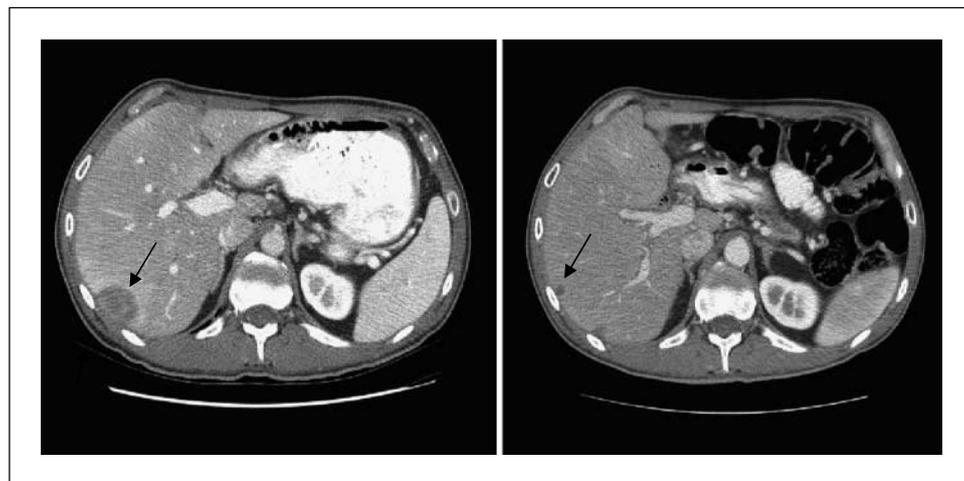

Fig. 2. Computed tomography scan of the abdomen showing hepatic lesions in patient 8. The computed tomography scans on the left were done pretherapy; the one on the right were done at 2 mo after starting curcumin. There was an overall 73% decrease in the size of liver lesions by Response Evaluation Criteria in Solid Tumors.

Table 2. Baseline cytokine levels and responses

Participant group	Baseline IL-6 level (pg/mL)	Baseline IL-8 level (pg/mL)	Baseline IL-10 level (pg/mL)	Baseline IL-1RA level (pg/mL)
Normal volunteers, median (range)	Undetectable (undetectable to 4.3)	Undetectable (undetectable to 33.0)	Undetectable (undetectable to 18.0)	275.0 (65.0-1,484.0)
Patients with pancreatic cancer, median (range)	5.9 (2.9-36.0)	30.6 (12.0-235.0)	13.4 (<3.9-25.0)	507.0 (275.0-1,757.0)
Responders				
Patient 8 (73% decrease according to Response Evaluation Criteria in Solid Tumors)	2.7	25.0	7.6	1,071.7
Patient 14 (stable for ≥12 months)	5.52	55.2	19.47	1,757.2

NOTE: The lower limits of the assay sensitivity were as follows: IL-6, 0.7 pg/mL; IL-8, 3.5 pg/mL; IL-10, 3.9 pg/mL; and IL-1RA, 22.0 pg/mL.

regulated by growth factors such as epidermal growth factor and because IL-6 is overexpressed in many tumors, including pancreatic cancer, plays a role in tumorigenesis, and can be regulated by curcumin (27). All 19 patients examined had activated STAT3 expression at baseline. There was a statistically significant decline ($P = 0.009$, Student's t test) in the percentage of pSTAT3-positive cells by immunocytochemistry after treatment with curcumin (Table 3; Fig. 4C).

Curcumin pharmacology. Plasma curcumin levels were determined in 19 patients, all of whom received a daily dose
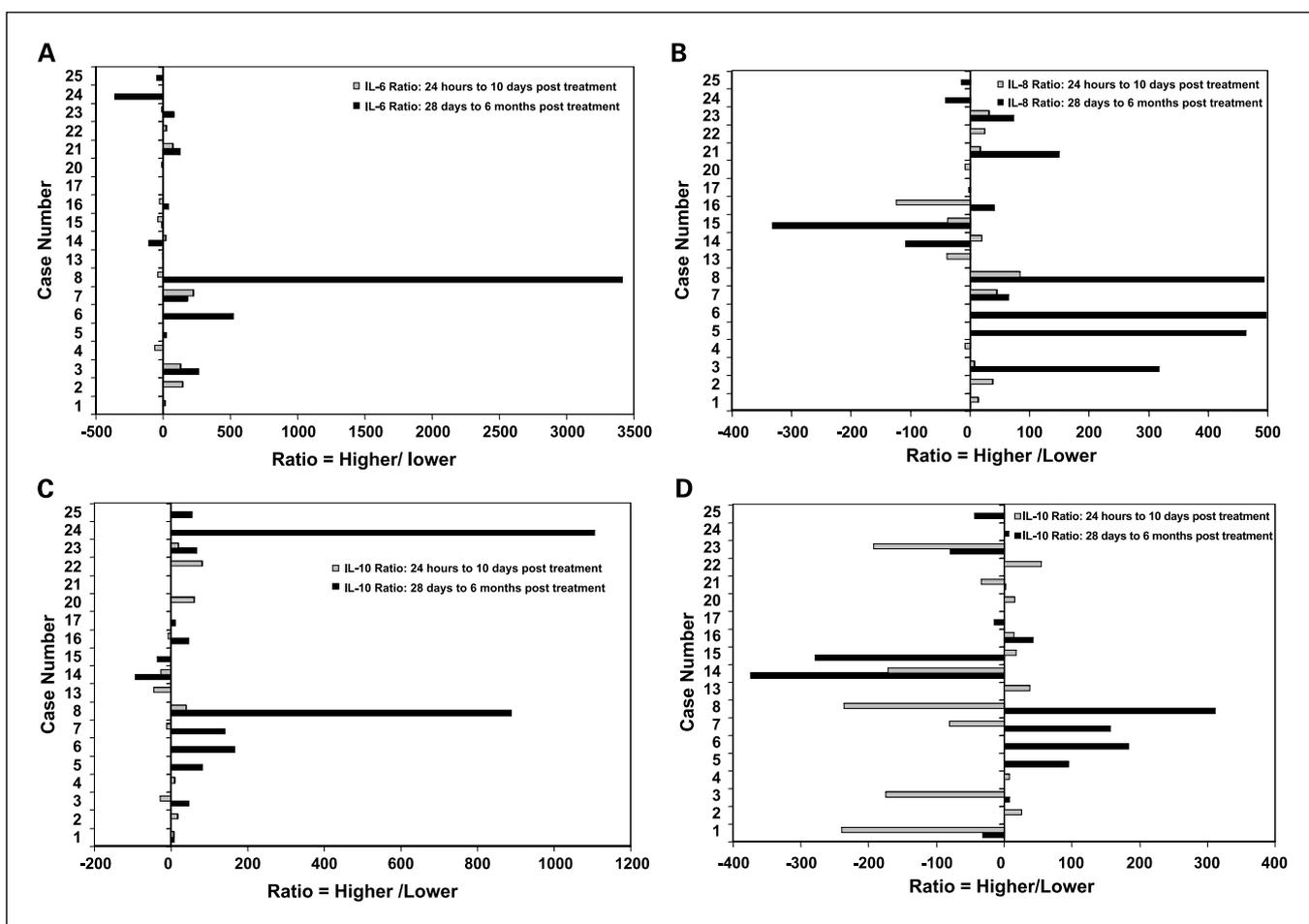

Fig. 3. *A* to *D,* ratio of cytokines IL-6, IL-8, IL-10, and IL-1RA respectively. Numbers are calculated by the following formula: (higher number / lower number × 100) - 100. If the pretherapy number was the higher number (cytokine levels decreased after treatment), a negative sign was given to the number calculated by the above formula. Cytokine levels were measured by ELISA as per methods. Of interest, patient 8 with the marked, albeit brief, response (depicted in Fig. 2) had a marked increase in cytokine levels after treatment. Patient 14 had stable disease for ≥12 mo (CA125 levels for patient 14 are shown in Fig. 1).
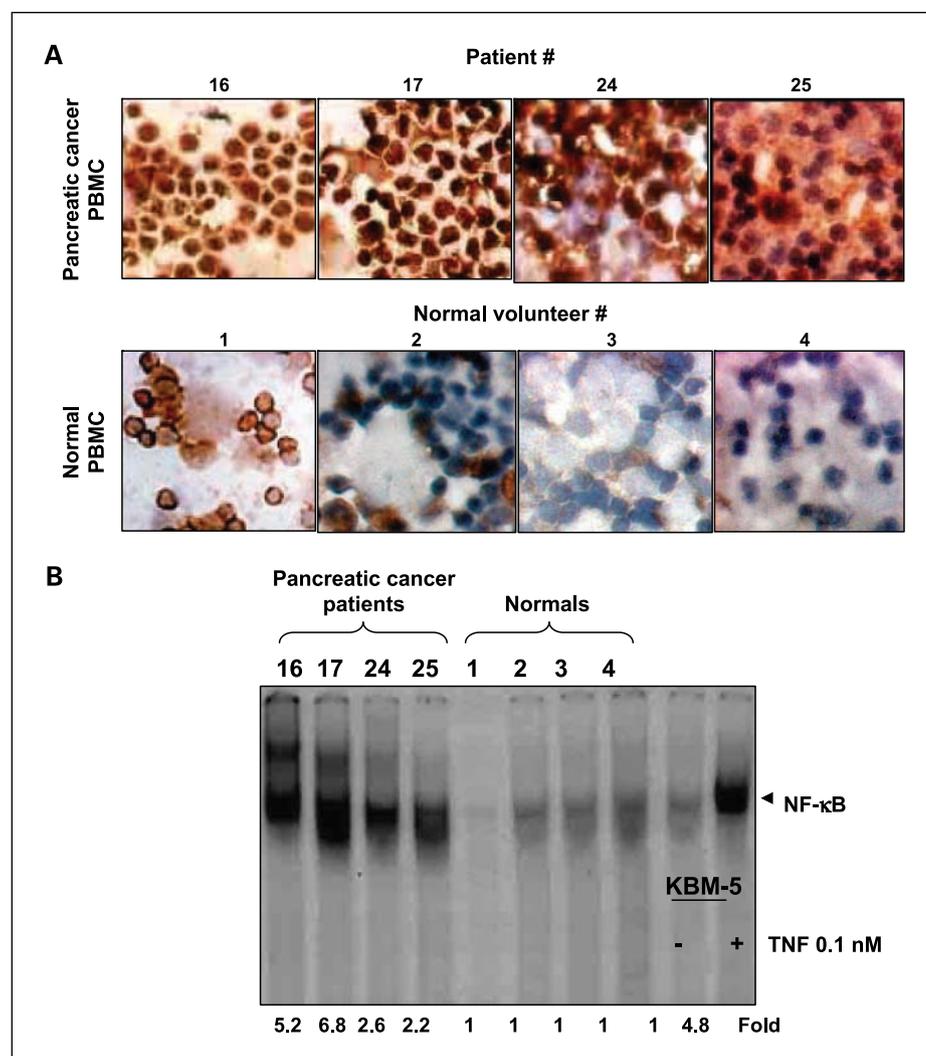
Fig. 4. *A,* PBMC from pancreatic cancer patients express constitutively activated NF-κB. Cells from patients at baseline (*top*) and from normal volunteers (*bottom*) were analyzed for nuclear p65 as described in Materials and Methods. There were high baseline levels of p65. *B,* PBMC from patients with pancreatic cancer show constitutive binding of NF-κB by EMSA, whereas PBMC from normal volunteers do not. KBM5 (myeloid leukemia line) with and without treatment with tumor necrosis factor (0.1 nmol/L for 30 min) were used as positive and negative controls (last two lanes). Nuclear extracts were prepared and analyzed for DNA binding by EMSA as described in Materials and Methods. Patients with pancreatic cancer showed high levels of constitutive NF-κB binding, whereas normal volunteers did not (results expressed as fold).

of 8 g curcumin. Although we found little, if any, free or unconjugated curcumin in these patients' plasma, we easily detected levels of curcumin following digestion of plasma with combined glucuronidase and sulfatase enzymes. This is consistent with data suggesting that curcumin is present in plasma in conjugated (glucuronide and sulfate) forms, thereby necessitating appropriate enzymatic hydrolysis before detection of free curcumin (24, 28). Plasma levels of drug released from conjugated derivatives of curcumin on day 1 of dosing decreased on average to 22 to 41 ng/mL from 2 to 6 h after the first dose of curcumin, although the C_{max} range across patients was considerable. For example, at 2 h, curcumin released from conjugate forms ranged from 0 (not detectable) to 125 ng/mL; at 24 h (before treatment on day 2), drug levels ranged from 1.8 to 117.0 ng/mL. The peak level in the patient with prolonged stable disease was only 2.6 ng/mL at 6 h, whereas the peak level in the patient with tumor regression was only 14.9 ng/mL. We found no evidence of a cumulative increase in drug levels throughout the 4-week sampling period. We detected an apparent steady-state level of conjugated curcumin in plasma that was achieved by day 3; this level was 22 to 41 ng/mL. Interestingly, three patients had small but

detectable levels of curcumin in their pretreatment plasma, suggesting that a dietary source of curcumin was already present.

Discussion

The anticancer potential of curcumin stems from its ability to suppress proliferation of a wide variety of malignant cell types, as well as tumor initiation, promotion, and metastasis, presumably due to its myriad biological properties (29). These properties include down-regulation of transcription factors such as NF-κB as well as other growth-regulatory molecules including, but not limited to, STAT3 and COX-2, cyclin D1, and growth factor receptors (such as epidermal growth factor receptor; ref. 30).

Based on our *in vitro* and *in vivo* (animal) work showing activity of curcumin and liposome-encapsulated curcumin in cell lines and models of pancreatic cancer (12, 13), and the fact that this activity was associated with down-regulation of NF-κB binding, we initiated the present study of oral curcumin. To date, this agent has been well tolerated, with no systemic toxic effects. We have seen antitumor effects in
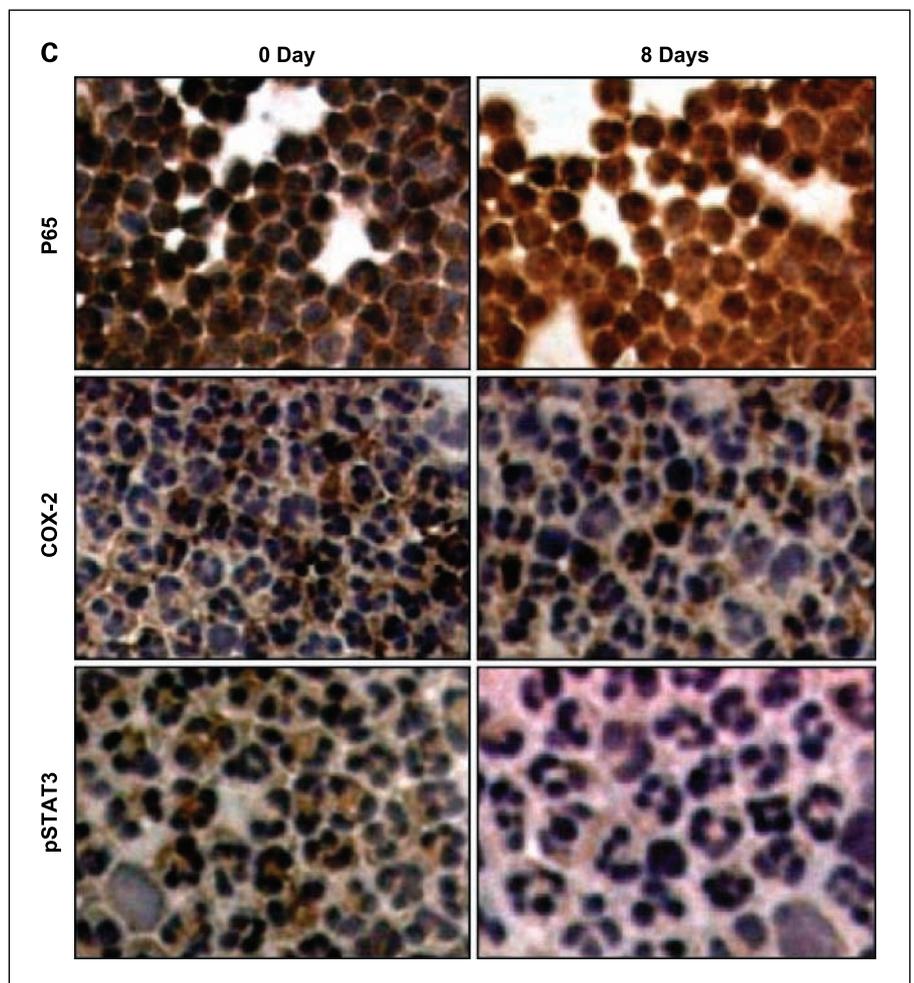
Fig. 4 *Continued. C,* representative example of a patient with pancreatic cancer who had baseline (day 0) increased expression of NF-κB (p65), COX-2, and pSTAT3 compared with the decrease in immunohistochemical staining after only 8 d of oral curcumin treatment.

two patients, one of whom had 73% tumor reduction (Fig. 2), which was, however, short-lived. Surprisingly, this patient had a rapid and dramatic increase in cytokine levels (IL-6, IL-8, IL-1RA, and IL-10; Fig. 3). Conceivably, this occurred because of release of cytokines from the tumor associated with shrinkage. Also, of potential importance in this patient is the observation that the tumors that originally regressed continued to show regression during the follow-up period on curcumin, whereas the tumors that grew were the ones that had been small originally. This observation suggests that there was a malignant clone responsive to curcumin, whereas another resistant clone emerged. In contrast, the patient who has appeared to have benefited most from treatment with curcumin (patient 14) has had slow improvement over 1 year and a gradual decrease in cytokine levels (Fig. 3). Of interest, patient 14 had the highest baseline levels of IL-1RA of any of the study patients. This may be biologically relevant because IL-1RA is a naturally occurring IL-1 antagonist, and we have seen *in vitro* growth promotion of pancreatic cancer cell lines by IL-1β.[6]

Cheng et al. (15) gave tablets containing curcumin to patients with premalignant conditions for as long as 3 months

and did not record any treatment-related toxic effects up to doses of 8 g/d. Beyond 8 g/d, the bulky volume of curcumin was unacceptable to the patients. Other studies have shown a similar lack of toxicity at daily doses of curcumin of up to 12 g (14–16, 23).

A key question related to treatment with curcumin is its poor bioavailability after being taken orally (23). Our results also indicated that only low levels of curcumin are detectable in plasma (steady-state level at day 3 is ~22-41 ng/mL). Nevertheless, some of the patients had biological activity of curcumin as evidenced by the antitumor effects noted above in two patients and by effects on cytokine levels and on NF-κB, COX-2, and pSTAT3, as described above. Conceivably, the limited bioavailability of curcumin attenuated the response rate, because exposure to microgram amounts of curcumin is required to show antiproliferative effects *in vitro* (12). It is also possible that circulating curcumin levels do not reflect tumor tissue curcumin levels.

Our results also showed that PBMC derived from almost all patients expressed constitutively active NF-κB (*n* = 18), whereas none of the PBMC from normal subjects did (*n* = 5; representative data in Fig. 4). NF-κB may have been activated in these patients because of high levels of cytokine expression, as multiple cytokines can induce NF-κB (20). Indeed, most of our patients had high baseline levels of IL-6, IL-8, IL-10, and

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[6] R. Kurzrock, manuscript in preparation.

Table 3. Expression of NF-κB (p65), COX-2, and pSTAT3 in PBMC derived from patients with pancreatic cancer by immunohistochemical staining

Patient no.	NF-κB (p65)*	NF-κB (p65) †	COX-2*	COX-2 †	pSTAT3*	pSTAT3 †
3	66	84	52	36	12	5
4	71	59	84	41	36	8
5	73	—	70	—	42	—
7	86	63	73	57	56	48
8	58	76	68	38	27	9
10	65	—	52	—	43	—
12	64	—	78	—	30	—
13	78	52	61	48	58	21
14	69	—	74	—	32	—
15	83	—	59	—	21	—
16	78	82	56	79	52	32
17	83	61	53	24	47	14
18	86	—	66	—	48	—
20	87	74	48	28	53	18
21	62	38	69	32	22	25
22	76	69	44	64	39	31
23	48	—	56	—	17	—
24	67	—	58	—	36	—
25	59	—	64	—	48	—

NOTE: Mean ± SD for p65, COX-2, and pSTAT3 pretreatment and posttreatment are 74.50 ± 10.00 and 65.80 ± 14.20 ($P = 0.131$), 60.80 ± 12.53 and 44.7 ± 17.37 ($P = 0.029$), and 40.20 ± 15.76 and 21.10 ± 13.30 ($P = 0.009$), respectively.
*Percentage of positive cells before treatment (by immunohistochemistry; an average of 300 cells was counted to make this determination).
†Percentage of positive cells after treatment (by immunohistochemistry; an average of 300 cells was counted to make this determination).

IL-1RA expression (Table 2). On the other hand, the high levels of NF-κB expression may have been responsible for the elevated baseline cytokine levels, because NF-κB can be found in the promoter regions of these cytokines and thus drives their expression. The expression of NF-κB correlated well with the expression of COX-2. This is not surprising as NF-κB is also a transcription factor for COX-2. The majority of the patients showed down-regulation of NF-κB and COX-2 after treatment with curcumin, but this down-regulation did not reach statistical significance for NF-κB (Table 3; representative data in Fig. 4C). This is the first study to show that curcumin can down-regulate the expression of these molecules in humans. However, the ability of curcumin to do this is consistent with earlier preclinical data from our group (9, 11, 13). Down-regulation of these factors was not associated with clinical response in many patients. It may be that down-regulation in PBMC does not reflect what occurs at the level of the tumor itself, and this may be one explanation for why many patients did not respond.

We also found that pSTAT3 was constitutively active in PBMC from almost all of the patients with pancreatic cancer (Table 3) but not in normal subjects. Curcumin treatment led to a decrease in constitutive pSTAT3 activation in most patients (Table 3; representative data in Fig. 4C). These results agree with reports from our group showing that curcumin can modulate pSTAT3 activation (22), a molecule implicated in tumorigenesis and chemoresistance (22, 31).

One important puzzle that arises from this study relates to why we see biological activity despite limited absorption and low nanogram levels of circulating curcumin. Low systemic bioavailability of curcumin after oral dosing is consistent with findings in preclinical models and in humans. It is now well established that curcumin exists in rodent and human plasma largely in conjugated forms, with the glucuronide conjugate present in much greater abundance than the sulfate conjugate (32). Little "free" or unconjugated drug is therefore typically found in plasma after oral dosing. Even plasma concentrations of curcumin released from conjugated forms, however, were surprisingly low. In the present study, these levels also varied widely among patients. It has been suggested previously that systemic levels of drug may not reflect drug levels actually present in tissues of interest (14). Although at least one study has examined curcumin levels in colon tissue of mice after oral administration (33), few, if any, studies have analyzed curcumin or curcumin metabolites in malignant human tissues. Furthermore, although all three forms of curcuminoids (curcumin, demethoxycurcumin, and bisdesmethoxycurcumin) have been shown to have important pharmacologic activity against malignant cell growth in vitro (34–37), few studies have reported the relative activity of curcumin glucuronide against malignant cell growth (38). Such information is, of course, of great importance as this form of curcumin represents the major circulating form of this drug. Therefore, further investigations are needed to elucidate the relationship between the form of curcumin, its relative pharmacologic activity, and circulating versus tumor tissue levels.

In conclusion, our current study shows that oral curcumin is tolerated without toxicity at doses of 8 g/d for up to 18 months. Although this molecule is poorly absorbed, with low nanogram levels of circulating curcumin detected at steady-state, biological activity is evident. Preclinical data suggest that curcumin has potent activity against pancreatic cancer (12, 13), but higher levels of exposure need to be achieved. Curcumin is hydrophobic and therefore cannot be given i.v. However, because it is lypophilic, it can be encapsulated in a liposome, and such a preparation would

allow i.v. administration, leading presumably to higher circulating levels of curcumin. We have reported previously that systemically administered liposomal curcumin has antitumor activity both *in vitro* and *in vivo* (13) and has no overt toxicity in animal models. Our current results suggest, therefore, that our plan to develop liposomal curcumin for clinical trials in cancer patients is a worthwhile strategy. This or other better formulations of curcumin may provide more consistent blood levels with better pharmacologic effect.

Disclosure of Potential Conflicts of Interest

V. Badmaev is employed by Sabinsa.

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Phase II Trial of Curcumin in Patients with Advanced Pancreatic Cancer

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