Short Communications

Clinical Development of Leukocyte Cyclooxygenase 2 Activity as a Systemic Biomarker for Cancer Chemopreventive Agents[1]

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Abstract

Advancement of cancer prevention and therapy requires clinical development of systemic biomarkers of pharmacological efficacy of the agent under scrutiny. Curcumin, a polyphenol derived from *Curcuma* spp., has shown wide-ranging chemopreventive activity in preclinical carcinogenic models, in which it inhibits cyclooxygenase (COX)-2 at the transcriptional level. COX-2 has been implicated in the development of many human cancers. To explore the inhibition of COX-2 activity as a systemic biomarker of drug efficacy, a biomarker of potential use in clinical trials of many chemopreventive drugs known to inhibit this enzyme, we measured COX-2 protein induction and prostaglandin E_2 (PGE_2) production in human blood after incubation with lipopolysaccharide (LPS). When 1 μM curcumin was added *in vitro* to blood from healthy volunteers, LPS-induced COX-2 protein levels and concomitant PGE_2 production were reduced by 24% and 41%, respectively ($P < 0.05$ by ANOVA). To test whether effects on COX-2 activity could also be measured after oral dosing in humans, we conducted a dose-escalation pilot study of a standardized formulation of *Curcuma* extract in 15 patients with advanced colorectal cancer. Basal and LPS-mediated PGE_2 production was measured in blood, twice pretreatment and on days 1, 2, 8, and 29 of treatment. Analysis of basal and LPS-induced PGE_2 production during treatment demonstrated a trend toward dose-dependent inhibition ($P < 0.005$ by regression analysis), but there was no significant difference compared with values from pretreatment time points. Measurement of leukocyte COX-2 activity should be considered in clinical trials of other agents likely to inhibit this isozyme.

Introduction

Curcumin (diferuloylmethane) is a polyphenol found in the dietary spice turmeric. This spice is derived from the dried rhizomes of the perennial herbs of *Curcuma longa* Linn. Curcumin has been shown to prevent cancer in the skin, forestomach, duodenum, and colon of mice and in the tongue, colon, mammary glands, and sebaceous glands of rats (1) and has been associated with regression of established solid malignancies in humans (2). Its chemopreventive activity has been demonstrated in both chemical (1, 3) and genetic (4) models of colorectal carcinogenesis.

In a previous study, we have shown that curcumin inhibits *COX-2*[3] induction in human colon cells by putative tumor promoters, in part through inhibition of nuclear factor κB activation at the level of the IκB kinase complex (5). Since induction of *COX-2* plays a role in colon carcinogenesis, this could represent an important mechanism of the cancer-preventive activity of curcumin (6). COX-2 has also been implicated in the pathogenesis of cancers of the breast, head and neck, lung, pancreas, stomach, and prostate (7). Systemic assessment of the effects of chemopreventive agents on COX-2 activity may therefore provide a surrogate indicator of the efficacy of intervention during carcinogenesis in several tissues.

Despite the low oral bioavailability of curcumin in the rat, we have shown that systemic levels of the parent drug may be improved by dissolution in an amphiphilic solvent (8), and other rodent studies of low-dose curcumin administered p.o. have shown that this compound is also active in preventing breast cancer and inhibiting induction of nitric oxide synthase activity in the liver (9, 10). Like *COX-2* transcription (5), the induction of *NOS* gene expression in macrophages involves the nuclear factor κB family of transcription factors (11). These findings suggest that despite its low bioavailability in animals, systemic assessment of curcumin's pharmacodynamic activity is indicated in humans.

Advancement of cancer chemoprevention requires pilot studies of promising agents with measures of pharmacokinetics and putative biomarkers of efficacy (1, 6). Such biomarkers should directly relate to the pharmacological mechanisms of the agent under scrutiny and may also act as surrogate markers of anticarcinogenic activity in the target tissue. Thus, inhibition of *COX-2* induction may serve as a useful marker of the systemic biological activity of curcumin. Previous studies have shown that whole blood can be used to measure inhibition of leukocyte COX-2 activity by drugs administered p.o. (12, 13). Although this method has been described (14) as "an accepted and reproducible standard" for measuring the effects of NSAIDs in healthy volunteers, it has thus far not been applied in cancer

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[3] The abbreviations used are: COX, cyclooxygenase; DL, dose level; NSAID, non-steroidal anti-inflammatory drug; PGE_2, prostaglandin E_2; LPS, lipopolysaccharide; BMI, body mass index.

chemotherapy or chemoprevention trials or to agents that inhibit COX-2 at the transcriptional level, such as curcumin.

To test the hypothesis that curcumin or other *Curcuma* extracts inhibit the COX-2 activity of blood leukocytes, we added curcumin or a standardized *Curcuma* extract to blood from healthy volunteers and measured PGE_2 production and COX-2 protein levels induced *in vitro*. To obtain evidence that the technique used here may prove useful in the assessment of systemic pharmacological activity of chemopreventive agents in clinical trials, we then tested the same hypothesis in a dose-escalation study of oral *Curcuma* extract in patients with advanced colorectal cancer.

Materials and Methods

Reagents. Acetylsalicylic acid, LPS, DMSO, and commercial grade curcumin were purchased from Sigma Chemical Co. (Poole, United Kingdom), and the selective COX-2 inhibitor NS-398 was purchased from Cayman Chemical Co. (Ann Arbor, MI). P54FP, an extract of *Curcuma* spp., was obtained as a liquid and in 220-mg capsules by donation from Phytopharm plc (Godmanchester, United Kingdom). High-performance liquid chromatography/mass spectroscopy analysis, by a method previously published with limit of detection 10 pmol for curcumin and its major metabolites (8), revealed that the Sigma Chemical Co. curcumin, which will be referred to as "curcumin" in the following text, contained 80% pure curcumin, 11% desmethoxycurcumin, and 9% bisdesmethoxycurcumin and that P54FP contained 9% curcumin and 1% desmethoxycurcumin, with the remainder constituted by essential oils derived from *Curcuma domestica* and *Curcuma xanthorrhiza*. The justification for use of this formulation has been published previously (15). Anti-COX-2 and anti-actin polyclonal antibodies and COX-2 protein standard were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antirabbit and antimurine horseradish peroxidase antibodies were purchased from Sigma Chemical Co.

Patients and Volunteers. The trial and its extensions were approved by the local ethics committee and the United Kingdom Medicines Control Agency. Blood from 10 healthy volunteers aged 20–40 years was used for *in vitro* studies. Patient characteristics and inclusion and exclusion criteria have been described previously (15). Patients and volunteers abstained from consumption of aspirin or NSAIDs and all foods containing turmeric while participating in the study, and three patients stopped taking NSAIDs at least 4 weeks prior to treatment. Written informed consent was obtained from each patient before enrollment.

Administration of P54FP to Patients. Each 220-mg capsule of P54FP contained 18 mg of curcumin and 2 mg of desmethoxycurcumin suspended in 200 mg of *Curcuma* essential oils. The curcuminoid content of each capsule was confirmed by high-performance liquid chromatography/mass spectroscopy, as described previously (15). There were 3 patients/DL. After at least 2 h fasting, patients consumed P54FP once daily with water at the following dose per day: (*a*) DL1, 2 capsules; (*b*) DL2, 4 capsules; (*c*) DL3, 6 capsules; (*d*) DL4, 8 capsules; and (*e*) DL5, 10 capsules. This scheme was based on the Fibonacci series currently used in Phase I chemotherapy trials, and there were no escalations or reductions of dose within each DL. Treatment was administered daily for at least 29 days.

Blood Sampling. All blood samples were collected in standard tubes pretreated with lithium-heparin (Sarstedt, Loughborough, United Kingdom), kept at 20°C, and treated within 30 min of collection. In all patients, basal and induced blood COX-2 activity was measured in samples taken during a screening visit 1 week before treatment and on days 1, 2, 8, and 29 of treatment immediately predose and 1 h postdose to investigate both immediate and cumulative dose effects.

Measurement of Basal and Inducible COX-2 Activity. We used a previously published method for the indirect assessment of monocyte COX-2 activity and gene expression in whole blood using plasma PGE_2 concentration and Western blotting (12). To irreversibly inhibit platelet COX-1 activity, acetylsalicylic acid (200 μM), which would have been rapidly degraded by enzymatic hydrolysis (12), was added to each 6-ml sample of whole blood. To determine whether curcumin inhibited *COX-2* induction *in vitro*, we immediately added curcumin (1–20 μM), P54FP (1–20 μM curcumin equivalent, see below), or NS-398 (10 μM), all of which were dissolved in DMSO, and identical volumes of DMSO were added to control samples. Each sample was incubated at 37°C for 30 min before the addition of LPS (0.1 or 10 μg/ml, as stated below) for *in vitro* induction of COX-2 activity (12, 13). The selective, competitive inhibitor of COX-2 catalysis, NS-398, was deemed a suitable positive control (13). Concentrations were based on published IC_{50} values and data obtained in colon cells grown *in vitro* (5, 16). P54FP was added to blood to give curcumin concentrations equivalent to those used in the experiments with curcumin. For treatment of blood samples from patients in the clinical trial, neither curcumin nor P54FP was added to blood, but the method was otherwise identical. After 3 h at 37°C, 4.5 ml of whole blood were removed, and leukocytes were isolated by Ficoll-Paque (Amersham Pharmacia Biotech, Bucks, United Kingdom) and resuspended in lysis buffer (5) before storage at −80°C. The remainder of the blood was incubated at 37°C for an additional 21 h, after which time plasma was separated by centrifugation and stored at −80°C for PGE_2 measurement by competitive enzyme immunoassay (Cayman Chemical Co.), with a detection limit of approximately 30 pg/ml plasma. All samples from each experiment with volunteer blood or all time points from one patient were analyzed on a single or at most two plates, and an internal standard was included on every plate. The coefficient of variation for repeat analyses of the same sample was less than 10%. Pretreatment with acetylsalicylic acid had no significant effect on the basal or LPS-induced level of PGE_2, as documented previously (12). COX-2 protein levels in leukocyte samples were assessed by Western blotting as described previously (5). Blots were stripped and reanalyzed for actin to control for protein loading and transfer. Because reanalyzing the blots for actin yielded insufficient signal for densitometry, 12-ml blood samples were used in experiments from healthy volunteers, but this increase in sample volume was not possible in patients enrolled in the clinical trial. Comparisons were made by densitometry using a laser scanning densitometer (Molecular Dynamics Co., Sunnyvale, CA).

Statistical Evaluation. Results were subjected to repeated measures ANOVA and *post hoc* Dunnett's comparison or regression analysis using Minitab software (Minitab Inc., State College, PA). Plots of residuals were used to ensure that the variances were homogeneous and that the residuals had a normal distribution. Because of the high degree of PGE_2 induction by LPS, basal and LPS-induced PGE_2 values were analyzed separately, and a log transformation of all of the LPS-induced values was performed. A value of $P < 0.05$ was considered to be statistically significant. Comparison of basal blood PGE_2 values (ng/ml) with BMIs (kg/m^2) for patient samples was performed by regression fit analysis.

Results

Effect of Curcumin on COX-2-mediated PGE_2 Production by Leukocytes *in Vitro*. We tested the hypothesis that curcumin alters stimulated PGE_2 production, as a measure of monocyte COX-2 activity (12). To that end, whole blood was incubated for 24 h with LPS (10 μg/ml) after a 30-min pretreatment with curcumin (1–20 μM). Addition of LPS caused a marked increase in plasma PGE_2 level ($P < 0.001$ by ANOVA) compared with basal control values (mean, 0.73 ± 0.06 ng/ml). Pretreatment with 1 μM curcumin caused a 24% inhibition of PGE_2 induction ($P < 0.05$ by ANOVA) relative to DMSO controls (mean, 26.28 ± 6.10 ng/ml), but the extent of this inhibition was reduced at 5 μM curcumin and abolished at 10 μM curcumin. To test the hypothesis that constituents derived from *Curcuma* spp. other than curcuminoids could alter the efficacy of curcumin to modulate COX-2 activity, P54FP, a standardized formulation of *Curcuma* extract, was added to whole blood to give curcumin concentrations equivalent to those used in the experiments with curcumin. In addition to curcuminoids, these incubates contained 10-fold higher concentrations of *Curcuma* essential oils (see above). The same degree of inhibition was observed (Fig. 1*A*), with no enhancement of COX-2 inhibition over curcumin alone. For comparison, experiments were also performed with the selective COX-2 inhibitor NS-398. Pretreatment with NS-398 (10 μM) caused 94% inhibition of LPS-mediated PGE_2 production, suggesting that changes in PGE_2 concentration were COX-2 mediated.

To make the results more relevant to the physiological concentrations of LPS observed in inflammation and cancer, parallel experiments were performed using 100-fold lower concentrations of LPS as a novel modification of the published method. Pretreatment with 1 and 5 μM curcumin caused 41% and 22% inhibition of PGE_2 induction, respectively ($P < 0.05$ by ANOVA for 1 μM curcumin), as shown in Fig. 1*B*. Addition of equivalent concentrations of *Curcuma* essential oils, the noncurcuminoid constituents of P54FP, without the curcuminoid components, did not cause inhibition in PGE_2 production (data not shown). Experiments studying the effect of submicromolar concentrations of curcumin were limited by the inhibitory activity of the DMSO solvent in this model system, and alternative solvents for curcumin were found to cause excessive leukocyte lysis.

Effects of Curcumin on Leukocyte COX-2 Protein Levels *in Vitro*. To determine whether changes in the production of PGE_2 correlated with changes in COX-2 protein levels, the latter were measured in leukocytes by Western blot. Although undetectable in the absence of LPS, incubation of whole blood for 3 h with LPS (10 μg/ml) caused a marked induction of COX-2 protein. Pretreatment with 1 μM curcumin caused a reproducible inhibition of this induction by approximately 24% ($n = 3$; borderline significance by ANOVA) relative to controls. Inhibition of COX-2 protein levels was not observed at higher curcumin concentrations.

Inducible PGE_2 in Blood from Patients Consuming *Curcuma* Extract. As part of a pilot clinical study of *Curcuma* extract in patients with advanced colorectal cancer (15), blood samples were obtained for assessment of basal and LPS-induced PGE_2 concentrations. BMI did not predict basal plasma PGE_2 values, despite such a relationship having been shown previously between BMI and rectal mucosal PGE_2 content in healthy volunteers with a history of resected colorectal polyps (17). LPS-induced plasma PGE_2 values were significantly higher than basal PGE_2 values at all time points measured ($P <$ 0.001 by ANOVA; see Fig. 2). Although LPS-induced PGE_2 values were approximately 20% lower in blood taken 1 h postdose compared with that taken predose, this difference was not significant. Similarly, there was no significant difference in basal PGE_2 between pre- and postdose values within each DL. Time-dependent trends related to treatment were not identified in basal or LPS-induced PGE_2 values.

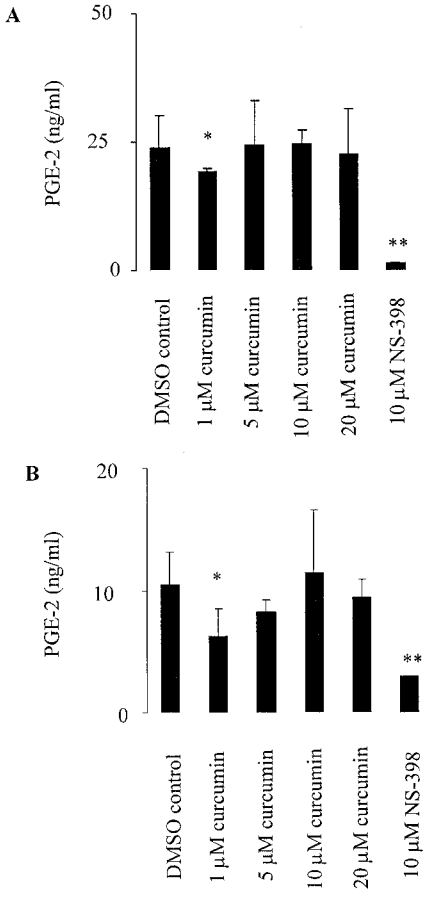

Fig. 1. Effect of P54FP (*A*) or curcumin (*B*) on LPS-induced plasma PGE_2 levels. Whole blood from healthy volunteers was incubated for 24 h in the presence of LPS (10 μg/ml in *A* and 0.1 μg/ml in *B*) after a 30-min pretreatment with P54FP (*A*) or curcumin (*B*) at concentrations defined by curcumin content (shown on the column labels). All results are the mean ± SD of three separate experiments. *Stars* indicate that the difference from control values is significant (*, $P < 0.05$; **, $P < 0.001$; assessed by ANOVA and *post hoc* Dunnett's comparison). For details of PGE_2 measurement, see "Materials and Methods."

Since there was no significant difference between pre- and postdose PGE_2 values, all time points after the first dose (*ie.* days 1, 2, 8, and 29) were combined to allow analysis of results from each DL for treatment-related effects. Values obtained 1 week before treatment and immediately before dosing on day 1 were combined to give "control" pretreatment values for the same patients. Plasma PGE_2 levels differed between DLs ($P =$ 0.006 by ANOVA), and the decrease in PGE_2 with increasing DL was confirmed by polynomial curve regression fit ($P <$ 0.005). Nevertheless, a comparable difference between DLs was also observed in "control" plasma samples (Fig. 2*A*). Pooled values for LPS-induced plasma PGE_2 from days 1, 2, 8, and 29 of treatment seemed to decrease in a dose-dependent fashion (Fig. 2*B*), but this trend was not significant ($P = 0.075$ by linear regression analysis).

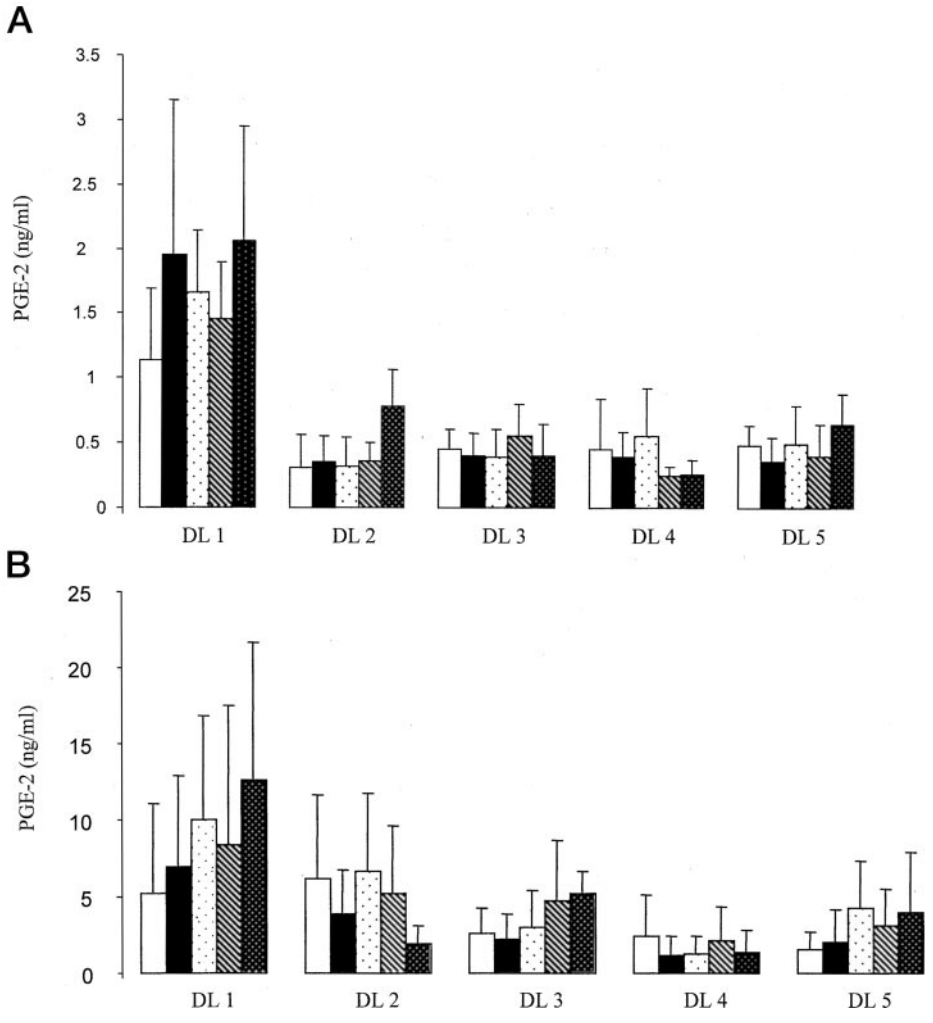

Fig. 2. Basal (*A*) and LPS-induced (*B*) PGE_2 levels in plasma of patients consuming P54FP capsules at the following daily doses of total *Curcuma* extract: (*a*) 440 (DL1); (*b*) 880 (DL2); (*c*) 1320 (DL3); (*d*) 1760 (DL4); and (*e*) 2200 mg (DL5), which contained (*a*) 36, (*b*) 72, (*c*) 108, (*d*) 144, and (*e*) 180 mg of curcumin. Blood was taken immediately predose or 1 h postdose on days 1, 2, 8, and 29. Whole blood was incubated for 24 h in the absence (*A*) or presence (*B*) of LPS (10 μg/ml). Results are the mean ± SD of triplicate measurements pooled from 1 week pretreatment combined with day 1 predose (□), day 1 postdose (■), day 2 pre- and postdose (*white bars with black dots*), day 8 pre- and postdose (▨), and day 29 pre- and postdose (*black bars with white dots*). For patient profiles and details of PGE_2 measurement, see "Materials and Methods."

Discussion

COX-2 is an important pharmacological target for NSAIDs, selective COX-2 inhibitors, and polyphenolic agents derived from the diet (6). Because COX-2 appears to play a pathogenic role in the carcinogenesis of many tissues, its pharmacological modulation holds implications for cancer prevention and treatment (6, 7). The results outlined above demonstrate that curcumin inhibits leukocyte *COX-2* gene induction and concomitant PGE_2 production when added to human blood *in vitro* and that measurement of this activity can be incorporated as a biomarker of drug efficacy in clinical trials of cancer-preventive or therapeutic agents that inhibit COX-2.

The inhibition of leukocyte COX-2 activity observed by adding 1 μM curcumin to whole blood, which was absent at higher curcumin concentrations, suggests that curcumin may possess pharmacological activity at physiologically relevant concentrations but that the therapeutic window may be narrow. Unfortunately, the limitations of the model system used here did not permit the study of submicromolar curcumin concentrations.

This study represents the first report of the measurement of blood COX-2 activity induced *ex vivo* as a biomarker of the efficacy of oral administration of an anticancer agent in a clinical trial. The findings of this pilot study demonstrate the feasibility of measuring this biomarker in patients with cancer, but they also highlight potential pitfalls. Previous studies have measured basal and LPS-induced PGE_2 values in healthy volunteers, reporting control levels comparable with those found here (12, 13), but the results of intervention have been compared with a single pretreatment time point. In the study reported here, we incorporated two pretreatment time points 1 week apart, and we discovered wide variation in both basal and LPS-induced PGE_2 values in patient samples, which rendered the significance of treatment effects invalid. Although some heterogeneity was attributable to interday variation within each patient, an even greater contribution arose from variation between patients, as had been observed previously for colon mucosal PGE_2 levels in a chemoprevention trial of ibuprofen in healthy individuals who had had polyps resected previously (17). To see treatment effects on blood PGE_2 production in the context of this variability, future prospective studies should stratify patient pretreatment according to measures of basal and LPS-induced PGE_2 concentration on at least two occasions, as has been suggested for trials measuring rectal mucosal PGE_2 levels (18). Such stratification of control and treatment groups by baseline PGE_2 levels could help balance subjects better and improve the chances of observing a treatment effect. Additional studies are indicated to determine the degree of inhibition of

PGE_2 production a COX-2 inhibitor must demonstrate in whole blood *in vitro* to justify measurement of this biomarker in clinical chemoprevention trials.

The clinical trial reported here is the first documentation of the low systemic bioavailability of oral curcumin in humans (15). Unless the bioavailability of curcumin is improved, as demonstrated in rats (8), large and perhaps impractical doses of curcumin may have to be consumed p.o. to achieve pharmacologically active levels in circulating monocytes. Moreover, recent preclinical studies in our laboratory would suggest that absorbed curcumin rapidly undergoes conjugation or reduction to metabolites with less COX-2-inhibiting potential than the parent compound (19). Despite its low systemic levels, large amounts of unaltered curcumin are observed in the colon mucosa of rats after oral dosing (8), which are potentially capable of altering COX-2 activity in this tissue and affecting tumor development in other rodent models (3, 4). We propose that the potential modification of COX-2 activity in colorectal adenomas by oral curcumin merits investigation, and such studies in a mouse model of familial adenomatous polyposis are ongoing in our laboratory.

In conclusion, because COX-2 is an important target for cancer chemoprevention, the systemic assessment of its pharmacological modulation may be a useful biomarker of drug efficacy. Such assessment may provide a surrogate measure of COX-2-inhibitory effects in the target tissue, depending on the bioavailability of the agent under scrutiny. The results of the pilot study of oral curcumin presented here suggest that measuring blood monocyte PGE_2 production may be a useful and feasible proposition in clinical trials of other putative chemopreventive agents that inhibit COX-2.

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References

1. Kelloff, G. J., Crowell, J. A., Hawk, E. T., Steel, V. E., Lubet, R. A., Boone, C. W., Covey, J. M., Doody, L. A., Omenn, G. S., Greenwald, P., Hong, W. K., Parkinson, D. R., Bagheri, D., Baxter, G. T., Blunden, M., Doeltz, M. K., Eisenhauer, K. M., Johnson, K., Knapp, G. G., Longfellow, D. G., Malone, W. F., Nayfield, S. G., Seifried, H. E., Swall, L. M., and Sigman, C. C. Strategy and planning for chemopreventive drug development: clinical development plans II. J. Cell. Biochem., *26S:* 54–71, 1996.

2. Kuttan, R., Sudheeran, P. C., and Josph, C. D. Turmeric and curcumin as topical agents in cancer therapy. Tumori, *73:* 29–31, 1987.

3. Kawamori, T., Lubet, R., Steele, V. E., Kelloff, G. J., Kaskey, R. B., Rao, C. V., and Reddy, B. S. Chemopreventative effect of curcumin, a naturally occurring anti-inflammatory agent, during the promotion/progression stages of colon cancer. Cancer Res., *59:* 597–601, 1999.

4. Mahmoud, N. N., Carothers, A. M., Grunberger, D., Bilinski, R. T., Churchill, M. R., Martucci, C., Newmark, H. L., and Bertagnolli, M. M. Plant phenolics decrease intestinal tumors in an animal model of familial adenomatous polyposis. Carcinogenesis (Lond.), *21:* 921–927, 2000.

5. Plummer, S. M., Holloway, K. A., Manson, M. M., Munks, R. J. L., Kaptein, A., Farrow, S., and Howells, L. Inhibition of cyclo-oxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-κB activation via the NIK/IKK signalling complex. Oncogene, *18:* 6013–6020, 1999.

6. Sharma, R. A., Manson, M. M., Gescher, A., and Steward, W. P. Colorectal cancer chemoprevention: biochemical targets and clinical development of promising agents. Eur. J. Cancer, *37:* 12–22, 2001.

7. Prescott, S. M., and Fitzpatrick, F. A. Cyclooxygenase-2 and carcinogenesis. Biochim. Biophys. Acta, *1470:* M69−M78, 2000.

8. Sharma, R. A., Ireson, C. R., Verschoyle, R. D., Hill, K. A., Williams, M. L., Leuratti, C., Manson, M. M., Marnett, L. J., Steward, W. P., and Gescher, A. Effects of dietary curcumin on glutathione *S*-transferase and malondialdehyde-DNA adducts in rat liver and colon mucosa: relationship with drug levels. Clin. Cancer Res., *7:* 1452–1458, 2001.

9. Pereira, M. A., Grubbs, C. J., Barnes, L. H., Li, H., Olson, G. R., Eto, I., Juliana, M., Whitaker, L. M., Kelloff, G. J., Steele, V. E., and Lubet, R. A. Effects of the phytochemicals, curcumin and quercetin, upon azoxymethane-induced colon cancer and 7,12-dimethylbenz[*a*]anthracene-induced mammary cancer in rats. Carcinogenesis (Lond.), *17:* 1305–1311, 1996.

10. Chan, M. M. Y., Huang, H. I., Fenton, M. R., and Fong, D. *In vivo* inhibition of nitric oxide synthase gene expression by curcumin, a cancer preventive natural product with anti-inflammatory properties. Biochem. Pharmacol., *55:* 1955–1962, 1998.

11. Xie, Q. W., Kashiwabara, Y., and Nathan, C. Role of transcription factor NF-κB/Rel in induction of nitric oxide synthase. J. Biol. Chem., *269:* 4705–4708, 1994.

12. Patrignani, P., Panara, M. R., Greco, A., Fusco, O., Natoli, C., Iacobelli, S., Cipollone, F., Ganci, A., Créminion, C., Maclouf, J., and Patrono, C. Biochemical and pharmacological characterization of the cyclooxygenase activity of human blood prostaglandin endoperoxide synthases. J. Pharmacol. Exp. Ther., *271:* 1705–1712, 1994.

13. Warner, T. D., Giuliano, F., Vojnovic, I., Bukasa, A., Mitchell, J. A., and Vane, J. R. Nonsteroidal drug selectivities for cyclooxygenase-1 rather than cyclooxygenase-2 are associated with human gastrointestinal toxicity: a full *in vitro* analysis. Proc. Natl. Acad. Sci. USA, *96:* 7563–7568, 1999.

14. Vane, J. R., and Warner, T. D. Nomenclature for COX-2 inhibitors (commentary). Lancet, *356:* 1373–1374, 2000.

15. Sharma, R. A., Gescher, A. J., McLelland, H. R., Ireson, C. R., Hill, K. A., Euden, S. A., Manson, M. M., Pirmohamed, M., Marnett, L. J., and Steward, W. P. Pharmacodynamic and pharmacokinetic study of oral *Curcuma* extract in patients with colorectal cancer. Clin. Cancer Res., *7:* 1894–1900, 2001.

16. Barnett, J., Chow, J., Ives, D., Chiou, M., Mackenzie, R., Osen, E., Nguyen, B., Tsing, S., Bach, C., and Freire, J. Purification, characterization and selective inhibition of human prostaglandin G/H synthase 1 and 2 expressed in the baculovirus system. Biochim. Biophys. Acta, *1209:* 130–139, 1994.

17. Chow, H. H. S., Earnest, D. L., Clark, D., Mason-Liddil, N., Kramer, C. B., Einspahr, J. G., Guillen-Rodriguez, J. M., Roe, D. J., Malone, W., Crowell, J. A., and Alberts, D. S. Effect of subacute ibuprofen dosing on rectal mucosal prostaglandin E_2 levels in healthy subjects with a history of resected polyps. Cancer Epidemiol. Biomark. Prev., *9:* 351–356, 2000.

18. Calaluce, R., Earnest, D. L., Heddens, D., Einspahr, J. G., Roe, D., Bogert, C. L., Marshall, J. R., and Alberts, D. S. Effects of piroxicam on prostaglandin E_2 levels in rectal mucosa of adenomatous polyp patients: a randomized Phase IIb trial. Cancer Epidemiol. Biomark. Prev., *9:* 1287–1292, 2000.

19. Ireson, C. R., Orr, S., Jones, D. L., Verschoyle, R., Lim, C. K., Luo, J. L., Howells, L., Plummer, S. P., Jukes, R., Williams, M., Steward, W. P., and Gescher, A. Characterization of metabolites of the chemopreventive agent curcumin in humans and rat hepatocytes and in the rat *in vivo*, and evaluation of their ability to inhibit phorbol ester-induced prostaglandin E_2 production. Cancer Res., *61:* 1058–1064, 2001.

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