

Effect of turmeric and curcumin on BP–DNA adducts

M.A.Mukundan, M.C.Chacko, V.V.Annapurna and K.Krishnaswamy¹

National Institute of Nutrition, Indian Council of Medical Research, Jamai-osmania, Hyderabad-500 007, India

¹To whom correspondence should be addressed

Many human cancers that are widely prevalent today can be prevented through modifications in life-styles, of which diet appears to be an important agent. Several dietary constituents modulate the process of carcinogenesis and prevent genotoxicity. Many plant constituents including turmeric appear to be potent antimutagens and antioxidants. Therefore the modulatory effects of turmeric and curcumin on the levels of benzo[*a*]pyrene induced DNA adducts in the livers of rats were studied by the newly developed ³²P-postlabelling assay method. Turmeric when fed at 0.1, 0.5 and 3% and the active principle of turmeric (curcumin) when fed at a level of 0.03% in the diet for 4 weeks significantly reduced the level of BP–DNA adducts including the major adduct dG-N₂-BP, formed within 24 h in response to a single i.p. injection of benzo[*a*]pyrene. The significance of these effects in terms of the potential anticarcinogenic effects of turmeric is discussed. Further, these results strengthen the various other biological effects of turmeric which have direct relevance to anticarcinogenesis and chemoprevention.

Introduction

Several human cancers appear to have a strong environmental component (1). It is now well accepted that dietary habits play an important role in a number of major human cancers (2). With new approaches to detect and treat cancer and better understanding of the process of carcinogenesis, cancer prevention and control appear to be feasible through modification of life-style factors (3). Besides the role of nutrients as major modulators of the carcinogenic process, several minor non-nutrient dietary constituents appear to be promising as chemopreventive agents (4,5).

As dietary habits are specific to populations and are varied, it is necessary to assess the antimutagenic or anticarcinogenic potentials of substances which are in common use. Since man gathered and cooked foods, spices have been used to make the food more appetizing as they add taste and aroma. Turmeric, a rhizomatous herb, its oleoresin and curcumin, the yellow pigment in turmeric, are all in use for their colour, flavour and medicinal properties (6). The major species of genus *curcuma* is *Curcuma longa* Linn, a predominant species yielding turmeric and curcumin of commercial value.

Using the Ames test, Nagabhushan and Bhide (7) have shown that turmeric is non-mutagenic and by itself can antagonize the mutagenicity of other substances. Further, Nagabhushan *et al.* (8) tested the effects of curcumin on mutagenicity induced by environmental agents and concluded that curcumin may alter the

metabolic activation and detoxification of mutagens. Our recent studies on *in vivo* mutagenicity in experimental animals and also human smokers (9,10) have revealed the strong antimutagenic potentials of turmeric. Apart from mutagenesis, carcinogen covalent binding to DNA is considered to be a key event in the initiation process (11). The objective of this paper is to examine the effects of turmeric/curcumin on DNA adducts analysed and quantified by the ³²P-postlabelling assay in experimental rats.

Materials and methods

All the materials used in this study were similar to those prescribed by Gupta *et al.* (12). The fine chemicals were obtained from Sigma Chemical Co., St Louis, MO. All other chemicals used were of highest purity available. Carrier-free [³²P]orthophosphate (100–150 mCi/ml) used for the synthesis of ATP was obtained from the Bhabha Atomic Centre, Bombay, India. MN-300 cellulose was obtained from Brinkman and Co., USA. Turmeric used in this study was obtained as rhizomes from a local market and was powdered and incorporated into a 20% casein diet. Curcumin was a generous gift from the Central Drug Research Institute, Lucknow, India.

Male rats of the Wistar/NIN strain weighing ~100 g were divided into seven groups of six animals each. The animals were fed on a stock diet containing 20% casein, 64% starch, 5% fat and vitamins and minerals mix with or without turmeric or curcumin. Groups I–III received neither turmeric nor curcumin while rats in groups IV, V and VI received turmeric at 0.1, 0.5 and 3% levels in the diet, respectively, and group VII received curcumin at 0.03% in the diet. The animals were housed in individual cages at 22 ± 2°C and allowed free access to their respective diets and water. They were maintained on a diurnal cycle of 12 h light/dark. At the end of 1 month, the rats in groups III–VII received a single i.p. injection of benzo[*a*]pyrene (BP*) (1 mg/kg body wt) in dimethylsulphoxide (DMSO). Rats in group I received no treatment while those in group II received DMSO (vehicle) alone. After 24 h of carcinogen/vehicle treatment the animals were killed by cervical dislocation and the livers were collected, washed in cold saline, blotted under folds of filter paper, packed in aluminium foil and stored at –60°C until use for isolation of DNA and analysis of adducts.

Isolation of DNA

DNA was isolated from the liver by solvent extraction after enzymatic digestion of protein and RNA (13). DNA concentrations were estimated spectrophotometrically using a value of 20 A₂₆₀ units of DNA per mg.

Digestion of DNA

A known aliquot of DNA was hydrolysed using micrococcal nuclease and spleen phosphodiesterase to deoxyribonucleoside 3'-monophosphates (12). An aliquot of this total DNA digest was diluted and used for the estimation of total nucleotides by one-dimensional TLC in the ³²P-postlabelling procedure. The remaining digest was used for the estimation of adducted nucleotides by the adduct enrichment procedure of Gupta (14). It involves extraction of the adducts with 1-butanol in the presence of the phase transfer agent tetra butyl ammonium ions.

Preparation of TLC sheets

Locally available X-ray sheets were coated with MN-300 cellulose. Prior to washing the sheets were subjected with dilute acid, alkali and petroleum ether. Final washing was done with distilled water. After drying, the sheets were coated with 5% polyethyleneimine–MN 300 cellulose slurry. After overnight drying the sheets were left in deionized water to remove impurities, followed by air drying at room temperature. The sheets were cut into appropriate sizes and used for the study. The sheets thus prepared were found to be as good as commercially available precoated sheets.

Synthesis of *γ*-³²P-labelled ATP

ATP was synthesized by the substrate level phosphorylation of ADP using carrier-free ³²P-orthophosphoric acid by the procedure of Johnson and Walseth (15). The ATP synthesized under these conditions was tested by running a one-dimensional TLC with 1.2 M LiCl and identified by autoradiography. The specific activity of ATP used in the present study was ~3000 Ci/mmol based on polynucleotide kinase catalyzed phosphorylation of a known amount of DNA

*Abbreviations: BP, benzo[*a*]pyrene; DMSO, dimethylsulphoxide; RAL, relative adduct labelling.

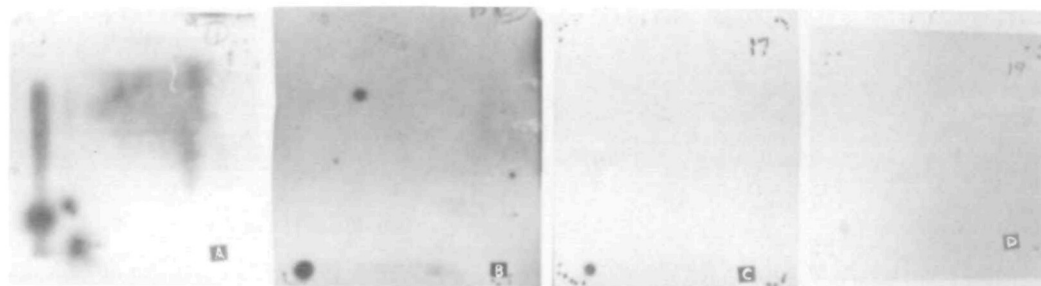


Fig. 1. DNA fingerprints of rats treated with A, BP at 1 mg/kg body wt; B, BP + 0.1% turmeric in the diet; C, BP + 0.5% turmeric in the diet; D, BP + 0.03% curcumin in the diet.

nucleotides. For labelling purposes ~100–120 μCi of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ was used for 10 μg DNA digest.

Labelling and quantification

^{32}P -Labelling of total nucleotides and the isolated adducts, the purification and resolution of ^{32}P -labelled adducts by multidimensional chromatography, autoradiographic detection, Cerenkov counting and calculation of relative adduct labelling (RAL) were done as described by Gupta (14).

Results

The ATP 32 synthesized to label the nucleotides when subjected to ascending PEI–cellulose TLC with 1.2 M LiCl had only one spot corresponding to ATP appearing near the origin indicating its purity. That ATP 32 did not stay at the origin with the adducts was verified by running the labelled ATP in one-dimensional TLC with 1 M sodium phosphate buffer (D_1 system for adduct purification). More than 99% of the ATP moved along with the solvent front.

Total nucleotides (normals) were eluted by low-salt elution (0.2 M LiCl) in one-dimensional TLC, under which $^{32}\text{P}_i$ migrated, leaving nucleotides at the origin. High salt (0.8 M ammonium formate) resolution of the digest showed no contamination at the origin and the bases were separated along with P_i , indicating complete DNA digestion and quantitative labelling of the resultant nucleotides.

Effect of turmeric and curcumin on BP adducts

When the DNA from rat liver treated with BP was analysed for BP–DNA adducts by the ^{32}P -postlabelling assay, several radioactive spots were noted (Figure 1A). In addition to the major adduct located diagonally to the origin, the sample exhibited a grey area extending from the origin to the upper left hand margin and the top extreme right end of the autoradiograms. No attempts were made in the present study to identify the major adduct by any standard procedures. The total RAL estimates were 41.08 fmol/ μg DNA or 1.37 adducts per 10^5 nucleotides (Table I). With 0.1% turmeric alone, the major adduct and the other radioactive spots disappeared leaving a fairly high intense spot at the origin (Figure 1B), and a less intense spot with 0.5% turmeric (Figure 1C). Further increase in turmeric level in the diet (3%) did not seem to have an additive effect (Table I). However, curcumin, the active principle of turmeric at a level of 0.03% in the diet, reduced remarkably the intensity of the spot at the origin as well as the other spots (Figure 1D). The total RAL was just 3.47 fmol/ μg DNA and is significantly lower than that of 0.1, 0.5 and 3% turmeric. Figure 2 shows the effect of different levels of turmeric and curcumin on BP–DNA adduct level as per cent of control values. Autoradiographs from untreated and vehicle treated rats showed a negligible number of spots.

Table I. Effect of turmeric and curcumin on BP–DNA adducts

No.	Treatment	RAL values (fmol/ μg DNA)	Mean no. of adducts per 10^5 nucleotides
1	Control BP treated	41.08 \pm 9.226 ^a	1.37 \pm 0.307
2	BP + 0.1% turmeric	15.91 \pm 2.077 ^{**b}	0.53 \pm 0.067
3	BP + 0.5% turmeric	9.72 \pm 4.848 ^{**c,e}	0.32 \pm 0.167
4	BP + 3% turmeric	12.11 \pm 8.173 ^{**b,c}	0.39 \pm 0.272
5	BP + 0.03% curcumin	3.47 \pm 2.453 ^{**d}	0.11 \pm 0.082

Values bearing different superscripts are significantly different (Mann–Whitney U–Wilcoxon rank test).

** P < 0.01, significantly different from control values.

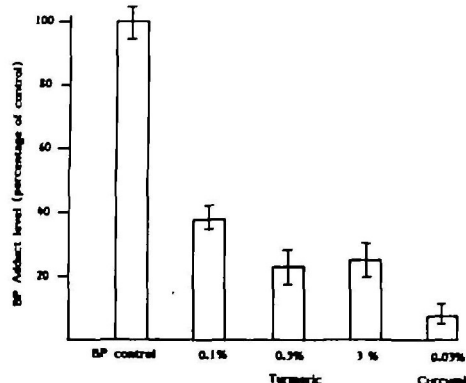


Fig. 2. BP–DNA adduct levels as a percentage of control values in turmeric and curcumin treated rats (means \pm SE).

Discussion

Carcinogenesis induced by chemicals, viruses, radiation, etc., involves separate and independent processes of initiation, promotion and progression (16,17). In theory, there are several possible ways in which diet can influence human carcinogenesis. Dietary substances that interfere with formation of carcinogens from precursors and those that decrease the binding of electrophilic substances with critical nucleophilic macromolecules, most importantly DNA, can be considered to be excellent blocking or inhibitory agents.

Epidemiological and experimental studies suggest an important role for dietary constituents in carcinogenesis. Chemoprevention with nutrients and non-nutrients as a prescriptive approach in addition to prescription is an attractive alternative in cancer control. Therefore, in the present study an attempt has been made to identify a naturally occurring anticarcinogen, among the dietary substances which is routinely used in the Indian cuisine.

One of the primary events in chemical carcinogenesis is the formation of covalent carcinogen–DNA adducts and as a

consequence of covalent interaction *in vivo*, point mutations may arise and initiate carcinogenesis in susceptible target tissues (18). Therefore using the ^{32}P -postlabelling assay which measures one adduct per 10^7 - 10^9 nucleotides, the effects of turmeric and curcumin on BP-DNA adducts have been quantified. Adduct enrichment with butanol extraction appears to be generally greater than with other enzymatic enrichment procedures. Rat livers treated with BP when analysed for BP-DNA adducts by the ^{32}P -postlabelling assay resulted in several radioactive spots (19). In addition to the major spot diagonal to the origin, several other spots were observed (20). It is obvious from the results that even as small a dose as 0.1% turmeric in the diet can significantly remove BP-DNA adducts in hepatic tissues. Dose responses, however, were not seen. At even 0.1%, the inhibition was >60%. Species and strain variation, the dose and the route employed and the time of killing do not permit us to compare our results with those documented in literature (21). Turmeric and curcumin totally inhibited the formation of the spot diagonal to the origin. Based on its migration on the TLC sheet it seems to be the major adduct dG-N₂-BP as reported previously (22) which is known to be responsible for mutagenesis and carcinogenesis (23).

It is even more interesting and encouraging to observe that as low as a concentration of 0.03% curcumin is more effective (>90% inhibition) than 0.1% turmeric. Curcumin by structure is a diferuloyl methane and is a phenolic derivative. Curcumin has been shown to be a strong antioxidant *in vitro* (24). Turmerin, a peptide recently isolated and characterized, also appears to be an efficient antioxidant (25). Our observations on curcumin, however, suggest that in addition to being a potent antioxidant, it can account for 90% inhibition of covalent binding. Further, our study on curcumin administered by oral route clearly shows that it is absorbed and is available for biological action whereas the *in vitro* efficacy of the peptide needs further evaluation under *in vivo* situations.

More recently, we have assessed the effects of turmeric on xenobiotic metabolizing enzymes which further attributes detoxifying properties to turmeric (26). Thus, turmeric, its active ingredient curcumin and probably its peptide component are all excellent inhibitors of the process of carcinogenesis. They may act as detoxificants, antioxidants and as anti-initiators. Bhide and co-workers (27) have documented inhibition of DNA single strand breaks in the forestomach tumours induced by BP. Our recent observations have demonstrated a protective effect of curcumin on DNA single strand breaks induced by genotoxicants in *Saccharomyces cerevisiae* suggesting enhancement of DNA repair process (in press). All these observations reinforce the anti-initiating effect of turmeric and are in keeping with inhibition of BP induced forestomach tumours and spontaneous mammary tumours in mice (28), skin tumours in mice (29) and anti-tumouricidal effects in culture cells (30).

From the experimental data presented in this and our previous studies, it is evident that turmeric at levels of 0.1-0.5% in the diet can decrease DNA adducts and mutagen load. An adult man weighing ~50 kg requires ~500 mg of turmeric per day as 0.1% is found to be active. Although the FAO/WHO expert committee on food additives estimated the acceptable daily intake of turmeric and curcumin up to 2.5 mg and 0.1 mg/kg body wt respectively (31,32), several recent studies demonstrated that turmeric or curcumin have no adverse effects even when used at high doses for long periods in experimental animals as well as in humans, under both *in vitro* and *in vivo* conditions (6,33-35).

Dietary interventions appear to be more attractive alternatives to chemoprevention as they can become part of our daily life-style changes. Spices in India are part of our regular diet and population surveys indicate that consumption of turmeric ranges between 0.2 and 0.6 gm/cu/day (36). Turmeric, by virtue of several mechanisms of anticarcinogenic activity, appears to be a promising dietary agent for use in humans. Randomized trials using short term definitive end points in subjects with precancerous lesions would perhaps establish the efficacy of this interesting dietary spice used in most South and South East Asian countries. Further studies are required to establish more clearly its efficacy in the human situation. Plants and plant products are perhaps the answer for prevention of several cancers.

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