Inhibitory effect of curcumin and its natural analogues on genotoxicity of heterocyclic amines from cooked food

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Curcumin (C) and its natural analogues demethoxycurcumin (dmC) and bisdemethoxycurcumin (bdmC), known for their potent anti-inflammatory, antioxidant, antimutagenic and anticarcinogenic effects, were tested for their possible inhibitory effects against seven cooked food mutagens (heterocyclic amines): 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoline (MeIQx), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-6-methylpyrido[1,2-a;3',2'-d]imidazol (Glu-P-1), in both TA98 and TA100 strains of Salmonella typhimurium using Ames Salmonellalreversion assay in the presence of Aroclor induced rat liver S9 homogenate. In the present investigations, curcumin as well as its two natural analogues i.e., dmC and bdmC were found to be highly effective in suppressing genotoxicity of all the tested cooked food mutagens in a dose-dependent manner, in both the frame shift (TA98) as well as base pair mutation sensitive (TA100) strains of S. typhimurium. However, bdmC appeared to be a relatively less active antimutagen compared to C and dmC. More than 80% inhibition of mutagenicity was observed at 200 µg/plate in case of C and dmC in both TA98 and TA100 against all tested cooked food mutagens. Whereas, bdmC showed 39-79% inhibition in TA100 and 60-80% inhibition in TA98, at a dose of 200 µg/plate. These findings warrant further biochemical, enzymatic and in vivo investigations in animal models as well as in humans to establish the chemoprotective effect of these agents against mutagenic heterocyclic amines found in cooked food.

Human beings are exposed to a unique class of dietary heterocyclic amine mutagens/carcinogens found exclusively in cooked foods. There is a growing concern about the role of these cooked food mutagens, which are generated at parts per billion level while cooking non-vegetarian foods like beef, chicken and fish at high temperatures, in the initiation of cancer. Several epidemiological studies published in recent years show a correlation between intake of fried meat and development of cancer and it has thus been hypothesized that these heterocyclic amines may induce cancer despite their presence in trace amounts in the food. The carcinogenic risk imposed by these probable human carcinogens is also modulated by other dietary factors that influence their uptake and bio-transformation. There is ample evidence that chemical mutagenesis and carcinogenesis can be inhibited by a large number of naturally occurring compounds of plant origin. These inhibitors are minor constituents of some commonly consumed vegetables, fruits, beverages and spices and the evaluation of antimutagenic potential of the chemical constituents of plants has been of recent interest especially because the data can be extrapolated to human cancer prevention. Therefore, it will be of interest to identify certain components of diet that could play a role in restricting the onset of carcinogenesis.

Turmeric obtained from dried powdered rhizomes of Curcuma longa L. (Zingiberaceae), has been used for centuries as a coloring agent in foods, drugs and cosmetics. The turmeric or its major yellow pigment curcumin is, at the moment, one of the most important chemoprotective agents under study for neoplasia prevention. Curcumin has been reported to possess high antioxidant and anti-inflammatory activities and also inhibitory activity against chemical induced carcinogenesis in several experimental models. It has also been shown to be a potent inhibitor of environmental mutagens requiring metabolic activation. Considering the reported antimutagenic action of curcumin and wide range use of turmeric as a very common household spice especially in India, it is appropriate to check the antimutagenic action of curcumin against any mutagens produced during cooking.

So far, these curcuminoids have been studied for suppressing the mutagenicity of chilly extract, capsicin, tobacco and cigarette smoke condensate, 2-acetamido-
flourene (2-AAF), benzo(a)pyrene (B[a]P), 7,12-dimethylbenz[a]anthracene (DMBA) and aflatoxin B1,14,17 in S. typhimurium but there is no report whether the different classes of heterocyclic aromatic amine mutagens are inactivated by curcumin and its analogues except for a very recent report in which only curcumin has been reported to be active against PhIP induced tumorigenesis in proximal Apc (min) mouse small intestine.18 Also it is not known as to what are the specific structural features in curcumin molecule responsible for its possible inhibitory activity against these mutagens. These food mutagens are promutagens/proximate carcinogens and require metabolic activation by cytochrome P450 monooxygenase enzyme system especially cytochrome P450 1A2.19,20 Curcumin has been shown to inhibit these Phase-1 microsomal enzymes in in vitro enzyme assays in the high-nanomolar to low-micromolar dose range and these levels are achievable in vivo even given the poor absorption kinetics of curcumin.21-23

Based on these literature studies, we carried out antimutagenicity testing of curcumin (C) and its natural analogues demethoxycurcumin (dmC) and bisdemethoxycurcumin (bdmC) using Ames Salmonella/mutagenic assay in two strains of Salmonella typhimurium namely TA98 and TA100 against various classes of heterocyclic amines found in human diet namely: amino imidazoazaarenes; IQ, MeIQ, MeIQx and PhIP; pyridoindole derivatives; Trp-P-1 and Trp-P-2; and dipyridoimidazole derivative; Glu-P-1.

Materials and Methods

Bacterial strains — A set of histidine requiring TA98 and TA100 strains of Salmonella typhimurium were obtained as a kind gift from Dr Bruce N. Ames (University of California, Berkley, USA).

Chemicals — 2-Amino-3-methylimidazo[4,5-f]quinoxaline (IQ), 2-amino-3,4-dimethylimidazo[4,5-f]quinoxaline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoline (MeIQx), 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2) acetate and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) were purchased from Toronto Research Chemicals Inc., Canada. 2-Amino-6-methylpyrido[1,2-a:3',2'-d]imidazole (Glu-P-1) hydrochloride (monohydrate) was purchased from Wako Pure Chemicals, Japan. 3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) acetate was kindly gifted by Dr T. Nohmi, National Institute of Hygienic Sciences, Tokyo, Japan. Curcumin (C), demethoxycurcumin (dmC) and bisdemethoxycurcumin (bdmC) were isolated from commercial turmeric powder by a method reported by Roughley and Whiting.24 Reference standards of these curcuminoids were received as a gift from Sami Labs Ltd, Bangalore, India. Albumin, bovine and nicotinamide adenine dinucleotide phosphate (NADP) sodium salt, were purchased from Sigma Chemical Company, USA. Oxyrase, nutrient broth No.2 was purchased from Oxoid Ltd., Basingstoke, Hampshire, England. Nutrient agar was purchased from Hi media Lab. Pvt. Ltd., India. All other reagents used were of AR grade.

Preparation of liver homogenate S9 fraction — The S9 fraction was prepared from the pooled liver homogenate of 2 male Sprague-Dawley rats previously induced with Aroclor 1254, by the method of Garner et al.25

Determination of protein concentration of 9— Protein concentration of induced rat liver S9 was determined by biuret method26 and it was found to be 54 mg/ml.

Antimutagenicity testing — The plate incorporation procedure given by Maron and Ames27 was used for antimutagenicity testing with the inclusion of preincubation step28 and with some minor modifications. The actual assay was as follows; 500 μl of S9 mix (containing 2.7 mg of protein; 50 μl of the optimum concentration when tested against 100 μg of plate of 2-aminofluorene), 100 μl of bacterial suspension (16 hr bacterial culture, 1-2 x 10⁉ cells/ml), 50 μl of antimutagen (C, dmC, bdmC) solution in DMSO and 50 μl of the respective mutagen solution (see Table 1 and 2 for dose) in DMSO, 2 ml of molten top agar, total volume 2.7 ml.

C, dmC and bdmC in the concentration range of 25-400 μg/ml were also tested for any toxic or mutagenic effects in both TA98 and TA100 strains and no change in spontaneous revertant count indicated absence of any mutagenic/toxic effects in the tested dose range.

A suitable dose of the test mutagens was selected from the linear portion of the dose-response curve of the respective mutagen for both TA98 and TA100. Further, mutagens were applied to the test in such doses which resulted in a maximum of about 2000 His⁺ revertants/plate (9-12-fold increase over spontaneous count), so as to ensure accurate counting, since at this count overlapping of bacterial colonies is avoided and inhibition or enhancement by modulators can be detected with a minimum statistical variation.
All assays were carried out in duplicate/triplicate on separate occasions. Results are expressed as mean ± SD of His+ revertants per plate (uncorrected for spontaneous count) for each dose.

**Statistical analysis**—All the data were statistically analysed by one way analysis of variance (ANOVA) followed by Student-Newman-Keuls method. The data which was not normal or where variations, were not equal, was subjected to Kruskal-Wallis one way analysis of variance (ANOVA) on ranks. Linear regression was used to test for linearity of dose-response relationship.

**Results**

**Effect of curcumin against mutagenicity of cooked food mutagens**—Curcumin in the nontoxic dose range of 50-200 μg/plate was found to effectively inhibit mutagenicity induced by all the seven tested cooked food mutagens in a dose-dependent manner in both TA98 and TA100 strains of *S. typhimurium* (Figs 1 & 2). A linear dose-response relationship was observed for all the mutagens tested (r=0.8-0.9), except for IQ in the TA100 strain (r=0.746). Further, the antimutagenic action of C was generally more profound in TA100 than in TA98 strain. The ID$_{50}$ values for IQ, MeIQ, MeIQx, Trp-P-1, Trp-P-2, PhIP and Glu-P-I being 137, 85, 86, 120, 120, and 98 μg/plate, respectively in TA98 and 33, 36, 118, 74, 48, 132 and 36 μg/plate, respectively in TA100 (Tables 1 & 2). The maximum inhibitory effect was observed against Trp-P-2 closely followed by that against MeIQ and MeIQx in TA98. In TA100, C was more effective against IQ, MeIQ and Glu-P-I and least effective against PhIP.

The mean values of His+ revertants per plate obtained at different doses of C in presence of mutagens were significantly different (P<0.05) from each other as well as from control i.e. the mean value of His+ revertant count in presence of mutagen alone; except in case of IQ, PhIP and Glu-P-I in TA98, where mean values at 50 μg/plate dose of C were not significantly different from control and in TA100 mean value at a dose of 100 μg/plate was not significantly different from mean value at 200 μg/plate dose.

**Effect of demethoxycurcumin against mutagenicity of cooked food mutagens**—Demethoxycurcumin, a natural analogue of C when tested for antimutagenic effects against various heterocyclic amines derived in S9-mediated TA98 strain of *Salmonella typhimurium*.

### Table 1 — Comparison of ID$_{50}$ values of curcumin and its natural analogues against Aroclor induced S9-mediated mutagenicity of heterocyclic amines in TA98 strain of *Salmonella typhimurium*

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>IQ (μg/plate)</th>
<th>MeIQ (μg/plate)</th>
<th>MeIQx (μg/plate)</th>
<th>Trp-P-1 (μg/plate)</th>
<th>Trp-P-2 (μg/plate)</th>
<th>PhIP (μg/plate)</th>
<th>Glu-P-I (μg/plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>137</td>
<td>85</td>
<td>86</td>
<td>120</td>
<td>80</td>
<td>120</td>
<td>98</td>
</tr>
<tr>
<td>Demethoxycurcumin</td>
<td>101</td>
<td>112</td>
<td>113</td>
<td>90</td>
<td>77</td>
<td>68</td>
<td>112</td>
</tr>
<tr>
<td>Bisdemethoxycurcumin</td>
<td>157</td>
<td>172</td>
<td>88</td>
<td>131$^*$</td>
<td>97</td>
<td>97</td>
<td>292</td>
</tr>
</tbody>
</table>

*ID$_{50}$ is the dose of antimutagen in μg/plate required to reduce the mutagenic activity of a given mutagen by 50%, calculated from corresponding dose response curves (Fig 1).  
*Value is extrapolated

### Table 2 — Comparison of ID$_{50}$ values of curcumin and its natural analogues against Aroclor induced S9-mediated mutagenicity of heterocyclic amines in TA100 strain of *Salmonella typhimurium.*

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>IQ (μg/plate)</th>
<th>MeIQ (μg/plate)</th>
<th>MeIQx (μg/plate)</th>
<th>Trp-P-1 (μg/plate)</th>
<th>Trp-P-2 (μg/plate)</th>
<th>PhIP (μg/plate)</th>
<th>Glu-P-I (μg/plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>33</td>
<td>36</td>
<td>118</td>
<td>74</td>
<td>48</td>
<td>132</td>
<td>36</td>
</tr>
<tr>
<td>Demethoxycurcumin</td>
<td>35</td>
<td>72</td>
<td>116</td>
<td>75</td>
<td>68</td>
<td>116</td>
<td>53</td>
</tr>
<tr>
<td>Bisdemethoxycurcumin</td>
<td>210</td>
<td>235$^{**}$</td>
<td>208$^{**}$</td>
<td>263$^{**}$</td>
<td>153</td>
<td>130</td>
<td>**</td>
</tr>
</tbody>
</table>

*ID$_{50}$ is the dose of antimutagen in μg/plate required to reduce the mutagenic activity of a given mutagen by 50%, calculated from corresponding dose response curves (Fig 2).  
*Values are extrapolated
**39% inhibition at 200 μg/plate (50% level of inhibition is not reached)
Fig. 1—Comparison of antimitogenic activity of C, dmc and bdmC against various cooked food mutagens in TA98 strain of Salmonella typhimurium. For the dose of mutagen see Table 1.
Fig. 2 — Comparison of antimutagenic activity of C, dmC and bdmC against various cooked food mutagens in TA100 strain of *Salmonella typhimurium*. For the dose of mutagen see Table 2.
from cooked muscle meats in TA98 and TA100 strains of *S. typhimurium*, exhibited strong inhibitory effects (Figs 1 & 2). These inhibitory effects were linearly related with the dose of dmC (r=0.8-0.9). The extent of inhibition obtained with dmC was closely similar to those observed with C. Like, C, a generally more potent antimutagenic effect was seen against all cooked food mutagens in TA100 as compared with its effect in TA98 (Tables 1 & 2). In TA98, very little antimutagenic activity was observed upto dose of 50 µg/plate, where as in TA100 a significant inhibition was seen at a dose of 25 µg/plate. The ID₉₀ values range between 77-113 µg/plate in TA98 and 35-156 µg/plate in TA100 (Tables 1 & 2). Highest inhibitory activity was observed against PhIP in TA98 and against IQ in TA100. Almost complete inhibition was achieved at dose level of 200 µg/plate.

The mean values of His⁺ revertant per plate obtained at different doses of dmC in presence of mutagens were significantly different (P<0.05) from each other as well as from control i.e. the mean value of His⁺ revertant count in presence of mutagen alone; except in case of IQ, MeIQ, MeIQx, Trp-P-1, Trp-P-2 and Glu-P-1 in TA98, where mean values at dose of 50 µg/plate were not significantly different from control and in case of MeIQ and Trp-P-1 in TA100 where mean values at a dose of 50 µg/plate were not significantly different from control. Also in case of Glu-P-1 and Trp-P-1 in TA100 mean values at a dose of 100 µg/plate of dmC were not significantly different from mean values at a dose of 200 µg/plate.

### Discussion

In this present investigation, we have tried to evaluate the antimutagenic potential of various constituents of turmeric(C, dmC, bdmC), a very commonly used dietary component, against the heterocyclic amine mutagens that are generated during cooking of muscle meats such as beef, fish and chicken, using a short-term genotoxicity assay i.e. Ames *Salmonella*/microsome assay. The Ames assay is well established and an excellent method for screening of such cancer chemopreventive factors.

Analysis of results of these studies as indicated in Tables 1 & 2 and Figs 1 & 2 clearly demonstrate that the three natural curcuminoids (C, dmC and bdmC) are highly effective in antagonizing the S9-mediated mutagenic effects of all the seven tested heterocyclic amines in a dose-dependent manner. In
general, C and dmC showed stronger inhibitory effects as compared to those of bdmC in both frameshift mutation sensitive TA98 and base pair mutation sensitive TA100 strain of *S. typhimurium* (Tables 1 & 2; Figs 1 & 2). More than 80% inhibition of mutagenicity was observed at 200 μg/plate in case of C and dmC in both TA98 and TA100 against all the tested cooked food mutagens. Where as, bdmC showed 39-79% inhibition in TA100 and 60-80% inhibition in TA98, at a dose of 200 μg/plate. Both C and dmC were found to exhibit greater antimutagenic effects in TA100 than in TA98 against all the tested cooked food mutagens (Tables 1 & 2).

Almost, similar pattern of activity and a potent antimutagenic effect shown by these natural curcuminoids against all the tested heterocyclic amines, strongly suggests the involvement of a similar mechanism of inhibition. These heterocyclic amines are reported promutagens/procarcinogens requiring metabolic activation for DNA-adduct formation. Numerous investigations have identified cytochrome P4501A2 as the catalytic enzyme essential for bioactivation of these heterocyclic amines. Curcumin is reported to inhibit various cytochrome P450 enzymes including cytochrome P4501A2 therefore, it may be proposed that the potent antimutagenic effects against cooked food mutagens obtained in our studies, may also be due to the inhibition of metabolic activation of these promutagens. Ineffectiveness of curcumin to inhibit the mutagenicity of direct acting mutagens: sodium azide, monoacetylhydrazine and streptozocin in TA98 and 4-nitrophenylenediamine in TA98 in absence of metabolic activation in Ames *Salmonella/microsome* test as reported by Nagabhushan et al., is further suggestive of involvement of curcumin in altering the metabolic activation of mutagens. Further, dmC and bdmC that structurally resemble curcumin may be proposed to act by the same mechanism.

Results of our investigations as shown in Tables 1 & 2 and Figs 1 & 2 indicate that curcumin was the most active inhibitor of genotoxicity of all the mutagens namely IQ, MeIQ, MeIQx, Trp-P-1, Trp-P-2 and Glu-P-1 except PhIP, when tested in TA100 strain of *S. typhimurium*, closely followed by dmC, while bdmC showed the least activity. Against PhIP in TA100, dmC showed a maximum inhibitory effect. However, in TA98 strain of *S. typhimurium*, C was most efficacious against MeIQ, MeIQx and Glu-P-1 followed by dmC and bdmC while dmC showed a higher activity against IQ, Trp-P-1, Trp-P-2 and PhIP. Thus, it may be suggested that presence of methoxy group on the benzene rings was responsible for high antigenotoxic effects of C and dmC as compared with bdmC molecule (structures shown in Fig. 3), in which both the methoxy groups on benzene rings, as found in curcumin, are replaced with hydroxy groups. Our experimental data is in concordance with the works of other authors. In these studies also presence of methoxy group has been shown to be responsible for higher antioxidant, anti-inflammatory and anticancer activities of C and dmC compared to bdmC.

To summarize, these findings indicate that the natural curcuminoids (C, dmC and bdmC) present in turmeric are very potent inhibitors of S9-mediated mutagenicity of heterocyclic amines. Since these are only preliminary investigations using *in vitro* bacterial system, it is very hard to predict whether similar effects can be expected in humans as complex mechanisms, namely, interactions with metabolic activation reactions are not adequately represented in *in vitro* assays with exogenous enzyme homogenates. However, this study does provide an impetus for the further evaluation of curcuminoids as possible chemopreventive agents in human cancers induced by these cooked food mutagens using the suitable mammalian cell lines and by carrying out biochemical, enzymatic and *in vivo* investigations in animal models as well as in humans.

**References**


