DOI:10.1021/jf900846n

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
CORRESPONDENCE/REBUTTAL

Comment on Curcumin Attenuates Acrylamide-Induced Cytotoxicity and Genotoxicity in HepG2 Cells by ROS Scavenging

Attenuation of toxicity induced by the neurotoxin acrylamide in HepG2 cells by curcumin dissolved in dimethyl sulfoxide (DMSO) (*1*) is an important step toward minimizing the hazards of acrylamide, especially in light of the presence of trace amounts of acrylamide in drinking water and the observation of acrylamide formation in potato chips and baked foods (*2*).

Interest in acrylamide, a monomer of polyacrylamide, spans the disciplines of soil science, plant science, environmental science, food science, microbiology, nutrition, pharmacology, toxicology, and medicine. In scientific research, acrylamide is used to selectively modify SH− groups in functional and structural proteins, as a quencher in structural tryptophan fluorescence studies, and as polyacrylamide gels to separate proteins (*2*).

Acrylamide has been implicated as a neurotoxin, genotoxin, and carcinogen in human and animal studies. Neurotoxicity has been attributed to either inhibition of kinesin-based fast axonal transport or the direct inhibition of neurotransmission. Acrylamide has been classified as a probable human carcinogen, the International Agency for Research on Cancer classifying it as 2A (*1*−*3*).

Recent reports of high acrylamide levels in heat-processed food and in carbohydrate-rich foods have been attributed to heat-induced reactions (Maillard reaction) between the amino group of asparagines and the carbonyl group of reducing sugars such as glucose during baking and frying. Foods rich in both of these sources are potatoes and cereals. Efforts to reduce acrylamide formation include suppression of biosynthesis of asparagines, use of asparaginase/amidase/acid to catalyze hydrolysis of asparagines to aspartic acid and ammonia, use of lower temperatures to process food, and lowering the pH of baking and frying formulations with citric acid (*2*).

Therefore, studies involving the use of curcumin to reduce acrylamide toxicity are a step in the right direction. Curcumin (1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione) is the yellow pigment that is the most active component in turmeric obtained from *Curcuma longa* (*4*) and has become the newest "nutraceutical" agent efficacious against colon cancer and other disorders as well as acting as an insect repellant and antimicrobial (*4*−*7*). In addition to inhibiting metastasis, tumorigenesis, platelet aggregation, inflammatory cytokine production, cataract formation, inflammatory bowel disease, and myocardial infarction, curcumin has been shown to lower cholesterol, suppress diabetes, enhance wound healing, modulate multiple sclerosis and Alzheimer's disease, and block HIV replication (*4*).

The major disadvantage in using curcumin for in vitro and in vivo assays is its aqueous insolubility and consequently its poor bioavailability. One study demonstrated (*8*) no detectable curcumin or its metabolites in the blood or urine following administration of 440−2200 mg of curcuma extract per day (36−180 mg of curcumin) for up to 29 days to patients with advanced colorectal cancer. Another study showed (*9*) that the peak curcumin levels in serum after administration of 4, 6, and 8 g of curcumin were 0.51, 0.64, and 1.77 μM, respectively. These investigators also showed that doses below 4 mg were barely detectable. Yet another group (*10*) found no curcumin in the serum of volunteers given 0.5, 1.0, 2.0, 4.0, 6.0, or 8.0 g of curcumin. However, these investigators found that curcumin levels reached 50.5 and 51.2 ng/mL of sera by 4 h in two subjects given 10 and 12 g of curcumin, respectively. Dhillon et al. showed that only about 22−41 ng/mL were detectable in plasma even when 8 g of curcumin/day was given orally (*11*).

Our laboratory has demonstrated heat-mediated increase in the solubility of curcumin (12-fold) and that of turmeric (3-fold). This was carried out by heating a solution of curcumin/turmeric in water to boiling for 10 min and centrifuging out the insoluble curcumin/turmeric. Profiling of the heat-extracted curcumin with matrix-assisted laser desorption ionization mass spectrometry and spectrophotometry (400−700 nm) showed no heat-mediated disintegration of curcumin (*12*, *13*). Curcumin solubilized by heat was found to inhibit oxidative modification of a peptide substrate by the lipid oxidation product 4-hydroxy-2-nonenal (HNE) by 80%, using an enzyme-linked immunosorbent assay (*14*) that used HNE modification of a solid-phase antigen substrate. Mild alkali solubilized curcumin was also shown to inhibit HNE protein modification significantly (*15*). Thus, inhibition of HNE modification may be a mechanism by which curcumin exerts its effect in many disorders (*12*, *15*).

Most of the curcumin (90%) dissolved in mildly alkaline pH (phosphate-buffered saline and serum-free media at 37 °C, pH 7.2) has been demonstrated to be broken down in 30 min (*4*). Heat treatment, however, appears to protect curcumin from breaking down more quickly. We studied the stability of heat-solubilized curcumin by storing it at 4 °C and measuring the optical density following centrifugation at 16000*g*. We found that heat-solubilized curcumin levels decreased by 47% in 12 h compared to starting amounts and by 67% in 72 h compared to starting amounts (*6*). Despite the fact that we found significantly enhanced solubility using heat, the bulk of the curcumin/turmeric was still insoluble (*6*).

Cao et al. (*1*) used curcumin solubilized in DMSO (final concentration of 0.1%) to see whether it would attenuate the toxicity of acrylamide on HepG2 cells. DMSO has been used as a solvent for chemotherapeutic drugs and has been used to treat rheumatic, pulmonary, gastrointestinal, neurological, urinary, and dermatological disorders because of its anti-inflammatory properties (*16*). The effects of DMSO on the outcomes of such studies are not completely clear yet. The concentrations of DMSO that have been reported to be safe vary considerably.

Published on Web 05/28/2009

There are data showing adverse effects of DMSO on the neuronal system. Hanslick et al. report (*17*) that DMSO induces apoptosis in a widespread manner in developing mouse cells at all ages tested, even at levels lower than that used in allogous bone marrow and organ transplantation (DMSO is used as a cryopreservative). In an in vitro experiment DMSO was found to induce neuronal loss at 0.5 and 1%.

One group studied the effects of very low concentrations of DMSO on the brain metabolism of [3-^{13}C]pyruvate and D-[1-^{13}C] glucose using ^{1}H/^{13}C NMR spectroscopy and a guinea pig cortical brain slice model. These investigators showed that DMSO accumulated in brain slices. DMSO was found to increase the metabolic rate, at all concentrations [0.000025−0.25% (v/v)] when [3-^{13}C]pyruvate was used as a substrate, as well as in the presence of D-[1-^{13}C]glucose at concentrations ranging from 0.00025−0.1% DMSO. These authors suggest that DMSO stimulates respiration and that there is no practical concentration of DMSO that can be used in metabolic experiments without effect (*18*).

It would have been of interest to see the effect of DMSO alone in Figures 1−4 in the paper by Cao et al. (*1*) as well as a dose−response effect of DMSO. On the basis of the published data described earlier, the biosafety of DMSO is clearly a questionable issue.

Quitschke added curcuminoids directly to human serum or curcuminoids dissolved in DMSO to human serum and found that both methods resulted in increased curcuminoid solubility in mammalian sera from different species (*19*). Soluble conjugates of curcumin have been demonstrated to exhibit enhanced cytotoxicity against certain cancer cell lines compared to the parent drug (*20*).

Our laboratory has shown that curcumin binds to albumin and several other proteins nonspecifically. We have used this property of curcumin to investigate antigen−antibody interaction. We found that heat-solubilized curcumin/turmeric significantly inhibited binding of auto-antibodies from Sjögren's syndrome (SS) (*21*) patients (up to 43/70%, respectively) and systemic lupus erythematosus (SLE) (*22*) patients (up to 52/70%, respectively) as well as an animal model (*23*) of SS (up to 50/60%, respectively) to their cognate antigens (*24*). In addition, we have used heat-solubilized curcumin as a nontoxic stain to detect proteins on SDS-PAGE gels as an alternative to the toxic Coomassie Brilliant Blue stain (*25*). However, it has to be noted that staining of proteins by curcumin was about 4−5-fold less sensitive than Coomassie Brilliant Blue staining (*25*).

Here, we suggest that the authors should solubilize curcumin with water and use the heat-solubilized curcumin for their in vitro assays, instead of solubilizing curcumin with DMSO for these assays (*26*). Curcumin has shown to be nontoxic to humans at 8 g/day for 18 months and can be used safely for in vivo assays as well. Even though it has been possible to increase the solubility of curcumin/turmeric, it remains to be known whether bioavailability in vivo would be increased.

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Received March 12, 2009. Supported by NIH Grants ARO49743 and ARO48940 to Dr. Hal Scofield, OMRF, OKC, OK.