Disparate effects of similar phenolic phytochemicals as inhibitors of oxidative damage to cellular DNA

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Abstract:

Phenolic phytochemicals are natural plant substances whose cellular effects have not been completely determined. Nordihydroguaiaretic acid (NDGA) and curcumin are two phenolic phytochemicals with similar molecular structures, suggesting that they possess comparable chemical properties particularly in terms of antioxidant activity. To examine this possibility in a cellular system, this study evaluated the capacities of NDGA and curcumin to function as antioxidants in inhibiting oxidative damage to DNA. Jurkat T-lymphocytes were pre-incubated for 30 min with 0–25 μM of either NDGA or curcumin to allow for uptake. The phenolic phytochemical-treated cells were then oxidatively challenged with 25 μM hydrogen peroxide (H_2O_2), Afterwards, cells were subjected to alkaline micro-gel electrophoresis (i.e. comet assay) to assess the extent of single-strand breaks in DNA. In a concentration-dependent manner, NDGA inhibited H_2O_2-induced DNA damage, whereas curcumin did not. In fact, incubating Jurkat T-lymphocytes with curcumin alone actually induced DNA damage. This effect of curcumin on DNA did not appear to reflect the DNA fragmentation associated with apoptosis because there was no proteolytic cleavage of poly-(ADP-ribose)-polymerase, which is considered an early marker of apoptosis. Curcumin-induced damage to DNA was prevented by pre-treatment of the cells with the lipophilic antioxidant, α-tocopherol, suggesting that curcumin damaged DNA through oxygen radicals. Therefore, it is concluded that NDGA has antioxidant activity but curcumin has prooxidant activity in cultured cells based on their opposite effects on DNA.

Keywords:

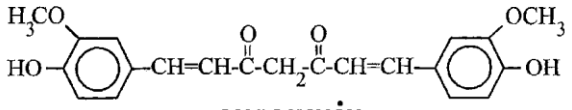
Antioxidants; Curcumin; DNA damage; Nordihydroguaiaretic acid; Phytochemicals

Article:

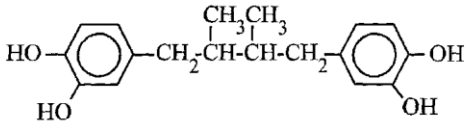
1. INTRODUCTION

The types of foods in our diets influence risk of several major chronic diseases. For example, diets rich in fruits and vegetables provide substantial amounts of phenolic phytochemicals that may reduce risk of cancer [1,2]. Multiple mechanisms are known by which phenolic phytochemicals may prevent development of cancer [2], but one mechanism is especially recognized. More specifically, because of their antioxidant properties, phenolic phytochemicals may inhibit oxidative damage to cellular DNA, and thereby, prevent mutagenesis and tumorigenesis. However, the full chemical properties and effects of phenolic phytochemicals have not been completely examined. In particular, it is unclear whether all phenolic phytochemicals function as antioxidants in protecting DNA against oxidative damage in a cellular system.

Two phenolic phytochemicals that have been widely studied are nordihydroguaiaretic acid (NDGA or 4,4'-(2,3-dimethyl-1,4-butanediyl)bis[1,2-benzenediol]) and curcumin (1,7-bis(4-hydroxy-3-methoxy-



curcumin



nordihydroguaiaretic acid (NDGA)

Fig. 1. Chemical structures of nordihydroguaiaretic acid (NDGA) and curcumin.

phenyl)-1,6-heptadiene-3,5-dione). NDGA is present in various plants, while curcumin is found in the rhizome of the plant species, Curcuma longa. Because of its brilliant yellow pigmentation, curcumin is customarily isolated for incorporation into the food spices, turmeric and curry. Both NDGA [3] and curcumin [4] have anti-tumorigenic activity, which may be partly due to their antioxidant properties.

From a molecular perspective, NDGA and curcumin have similar chemical structures (Fig. 1). The active centers of NDGA and curcumin are likely the phenolic hydroxyl groups. Such structural substituents can furnish hydrogen atoms and/or chelate metal ions in enabling the phenolic phytochemicals to function as antioxidants [5]. As such, it is anticipated that both NDGA and curcumin can inhibit DNA damage in cells subjected to oxidative stress. This possibility was investigated in the present study, which yielded results contradictory to what would be expected.

2. MATERIALS AND METHODS

2.1. Materials

The agarose for electrophoresis, as well as curcumin, NDGA, α-tocopherol and all other reagents, were purchased from Sigma Chemical Co. (St. Louis, MO). Frosted microscope slides were purchased from Fisher Scientific Co. (Pittsburgh, PA). Rabbit polyclonal antibody to poly-(ADP-ribose)-polymerase (PARP) was purchased from Boehringer Mannheim Corp. (Indianapolis, IN). Goat anti-rabbit secondary antibody/horseradish peroxidase conjugate and luminol reagent were both purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Jurkat T-lymphocytes, which are a human leukemia cell line, were purchased from ATCC (Rockville, MD).

2.2. Cell culture and treatment

Jurkat T-lymphocytes were maintained at 50–70% confluency in a humidified CO_2 incubator (95% air and 5% CO_2) set at 37°C. Culture media was RPMI-1640 containing 10% FBS. About 2.5 x 10^4 cells per 0.5 ml media were pre-incubated for 30 min with 0–25 μM of either NDGA or curcumin in 12-well Falcon tissue culture plates to allow uptake of the phenolic phytochemicals. The phenolic phytochemicals dissolved in absolute ethanol were added to the cell suspensions to give a final ethanol concentration of 0.2%, a level also present in control cells not treated with phenolic phytochemicals. After harvesting the cells following the pre-incubation, the cells were washed twice and resuspended in ice-cold, phosphate-buffered saline (PBS). Hydrogen peroxide (H_2O_2) was added to the cell suspensions to give a final concentration of 25 μM. Cells were then incubated for 30 min on ice to minimize DNA repair before assessment of DNA damage as described below.

In other experiments, Jurkat T-lymphocytes were pre-incubated with 100 μM α-tocopherol for 24 h at 37°C. The cells were washed and then resuspended in PBS for subsequent incubation with 0–5 μM curcumin for 30 min on ice before assessment of DNA damage. Additionally, Jurkat T-lymphocytes were incubated with 0–100

μM of either NDGA or curcumin by themselves for either 30 min or 20 h depending on the experiment, and then immediately analyzed.

2.3. Determination of reactive oxygen species in cell culture medium

It was recently reported [6] that adding various phenolic phytochemicals to cell culture medium results in the generation of H_2O_2 and that this could contribute to the effects of the phenolic phytochemicals on cells. Therefore, to similarly examine if curcumin and NDGA have the capacity to promote H_2O_2 generation, 0–100 μM of each test compound was added separately to complete RPMI-1640 medium and then incubated for 30 min. Epigallocatechin gallate, which is another phenolic phytochemical, was used as a positive control because it is a known inducer of H_2O_2 production [6]. Afterwards, the concentrations of H_2O_2 were measured using the ferrous ion oxidation-xylenol orange (FOX) reagent [6]. That is, 0.9 ml of FOX reagent was added to 0.1 ml of the samples or H_2O_2 standards, followed by mixing. After 30 min, tubes were centrifuged at 16,000 x g. The absorbance of the supernate was measured at 560 nm.

2.4. Detection of DNA single-strand breaks

To detect cellular DNA damage as single-strand breaks, alkaline micro-gel electrophoresis (comet assay) was performed essentially as described by Singh and coworkers [7]. Cells (2.5×10^4) were pelleted by centrifugation at 300 x g. The pellet was washed and resuspended in 85 μl PBS. The cell suspension was then mixed with 85 μl of 1% low gelling temperature agarose (Type VII from Sigma) dissolved in PBS. Next, 75 μl of this cell–agarose mixture was transferred onto a frosted microscope slide that had been pre-coated with 85 μl of 1% normal gelling temperature agarose (Type I from Sigma). A third layer of 0.5% low gelling temperature agarose (75 μl) was applied over the second layer containing the cells. In establishing each of the three layers of agarose, glass coverslips were applied on top of the liquid agarose to spread the agarose across the surface of the slides. Each layer of agarose was congealed by placing the slides on a metal tray which was positioned in crushed ice.

After removing coverslips, the slides were immersed slowly in ice-cold, alkaline cell lysis solution (2.5 M NaCl, 0.1M disodium EDTA, 0.3 M NaOH, 1% Triton X-100, 10% DMSO, 10mM Tris, pH 10) and allowed to incubate for 1 h. Slides were then placed carefully in an EC 360M horizontal electrophoresis unit (E-C Apparatus, Holbrook, NY) containing 0.3 M NaOH/1 mM disodium EDTA solution and left undisturbed at room temperature for 20 min. Next, electrophoresis was performed at 20 V (300 mA) for 20 min. Afterwards, slides were gently immersed in neutralization buffer (0.4 M Tris, pH 7.5) for 5 min, and this step repeated. After applying 60 μl ethidium bromide solution (20 μg/ml) on the top of the agarose and covering with coverslips, the slides were viewed with a Zeiss Axioplan fluorescence microscope with MC 100 camera attachment. Representative views of the slides were photographed, and the slides were examined to assess the relative extent of cellular DNA damage [8]. That is, 50 randomly selected cell nucleoids were scored on a scale of 0–4 to give the overall comet score ranging from 0 (no DNA damage) to 200 (severe DNA damage). While subjective, this scoring method is consistent with computer imaging analysis [8].

2.5. Detection of possible apoptosis induced by the phenolic phytochemicals

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)/Western-immunoblotting analysis was performed to detect any proteolytic cleavage of poly-(ADP ribose)-polymerase (PARP), which is an early definitive marker of apoptosis [9]. After washing in PBS and pelleting by centrifugation, the cells from above were resuspended in PBS containing 10 mg/ml phenylmethylsulfonyl fluoride and lysed by sonication. Protein concentration of the cell lysates was determined with a Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Hercules, CA). Aliquots of cell lysates (20 μg protein) were electrophoresed using 4–15% gradient gels (Novex, San Diego, CA). Afterwards, proteins were electroblotted to nitrocellulose membrane that was subsequently blocked with 5% nonfat milk. For immunodetection, the membrane was incubated with rabbit anti-PARP antibody and then with goat anti-rabbit secondary antibody conjugated to horseradish peroxidase. Chemiluminescent signal was generated using luminol reagent and simultaneously captured on X-ray film.

3. RESULTS

In the comet assay to detect cellular DNA damage [7,8], DNA within the nucleoid from each cell typically appears as either an intact spherical mass (i.e. no DNA damage) or a "comet" (i.e. DNA damage) upon staining with ethidium bromide and viewing by fluorescence microscopy. A basic assumption is that the overall span of the tail region of the comets is a general indication of the extent of DNA single-strand breakage. The photographs shown in the figures below are representative views of the slides for untreated and treated cells. The number and overall tail spans of the comets were found to be consistently similar in viewing different areas of each slide upon replicating the experiments three times and generating the overall comet scores that reflect the relative extents of DNA damage.

Fig. 2 shows the effects of increasing concentrations of NDGA in preventing DNA damage in Jurkat T-lymphocytes challenged with H_2O_2. In contrast to control cells (panel A, comet score: 12 ± 3), cells treated with H_2O_2 were characterized by clearly discernible comets (panel B, comet score: 187 ± 13). However, pre-incubating cells with as little as 1 μM NDGA and then treating them with H_2O_2 resulted in noticeable reduction of the overall comet tail spans, and hence, damage to DNA (panel C, comet score: 160 ± 9). Moreover, 5 and 10 μM NDGA had additional protective effects against DNA damage (panels D and E, comet scores: 119 ± 16 and 49 ± 8, respectively). No distinct comets or significant DNA damage were found in cells pre-incubated with 25 μM NDGA and then oxidatively challenged (panel F, comet score: 16 ± 4).

In the case of curcumin, unexpected results were obtained. Curcumin was unable to inhibit DNA damage induced by H_2O_2 (data not shown). Instead, incubating Jurkat T-lymphocytes with curcumin alone actually caused the formation of comets. This effect by curcumin was in a concentration-dependent manner (Fig. 3), suggesting higher intracellular concentrations of curcumin as the Jurkat T-lymphocytes were incubated with increasing concentrations of curcumin. In support of this suggestion, the research of others [10] has shown that incubating rat peritoneal macrophages with 5, 10, and 50 μM curcumin resulted in intracellular concentrations of 4.62, 8.82, and 44.35 μM, respectively, thus establishing about 90% uptake of curcumin by the cells. Emergence of distinct comet tails was evident after the comet assay of the Jurkat T-lymphocytes that had been incubated with only 1 μM curcumin (panel C, comet score: 79 ± 10), when compared to the comet assay of either untreated control cells (panel A, comet score: 7 ± 2)

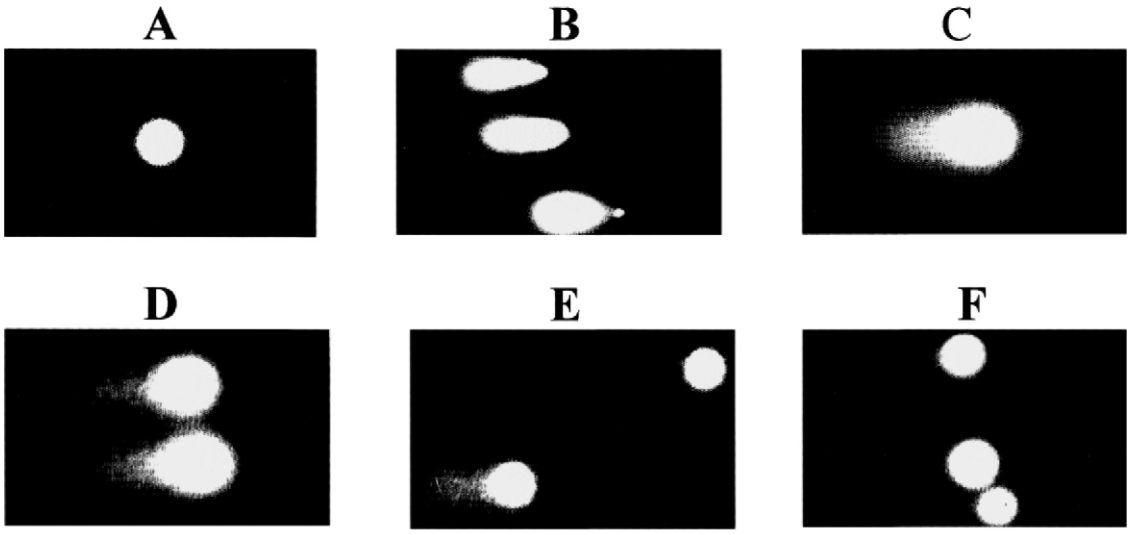


Fig. 2. Protective effect of nordihydroguaiaretic acid (NDGA) against single-strand breakage of DNA in Jurkat T-lymphocytes challenged with hydrogen peroxide (H_2O_2), as evaluated by the comet assay. Cells in complete medium were pre-incubated with NDGA for 30 min in a CO_2 incubator at 37°C. The cells were washed and resuspended in phosphate-buffered saline, before being incubated with H_2O_2 for 30 min on ice. The comet assay was then performed. Panel A: untreated control; panel B: 0 μM NDGA/25 μM H_2O_2; panel C: 1 μM NDGA/25 μM H_2O_2; panel D: 5 μM NDGA/25 M H_2O_2; panel E: 10 μM NDGA/25 μM H_2O_2; panel F: 25 μM NDGA/25 μM H_2O_2.

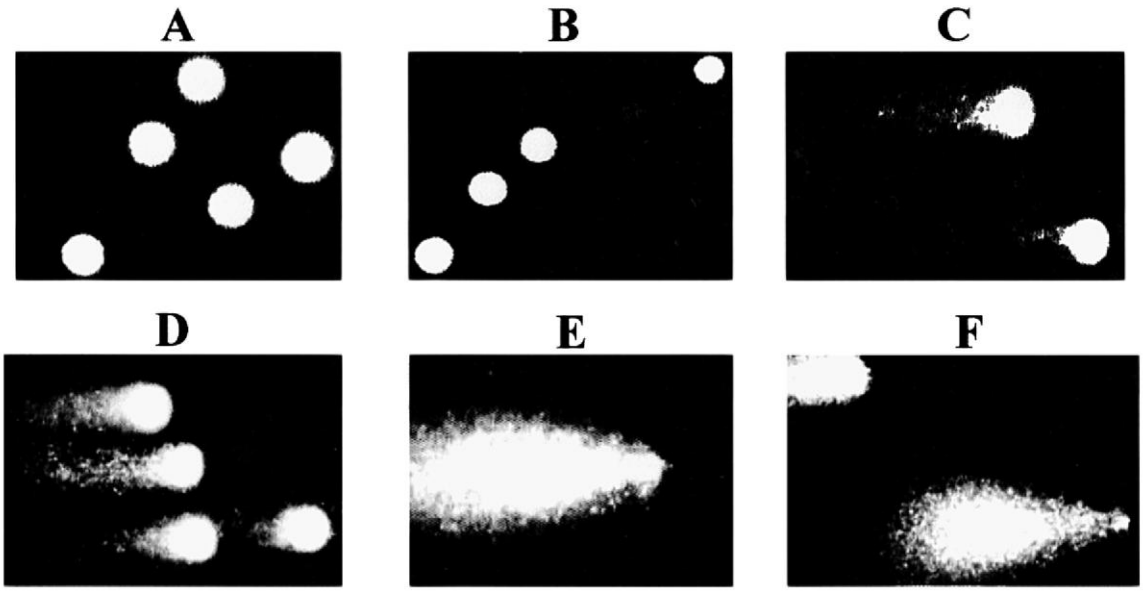


Fig. 3. Single-strand breakage of DNA in Jurkat T-lymphocytes treated with curcumin, as evaluated by the comet assay. Cells in phosphate-buffered saline were incubated with curcumin (dissolved in ethanol at 0.2% in the total incubation volume) for 30 min on ice. The comet assay was then performed. Panel A: untreated control; panel B: ethanol control; panel C: 1 μM curcumin; panel D: 5 μM curcumin; panel E: 10 μM curcumin; panel F: 25 μM curcumin.

or ethanol vehicle control cells (panel B, comet score: 13 ± 9). More pronounced comets were detected in cells that had been incubated with 5 μM curcumin (panel D, comet score: 101 ± 18). Cells that had been incubated with 10 and 25 μM curcumin (panels E and F, comet scores: 181 ± 14 and 194 ± 9, respectively) showed comets without "heads" but only "tails", indicative of much more extensive DNA single-strand breaks. In total contrast, short-term incubation of Jurkat T-lymphocytes with NDGA did not result in the appearance of comets (data not shown).

In addition to detecting oxidative DNA damage, the comet assay is capable of detecting endonuclease-mediated fragmentation of DNA that occurs during apoptosis [11]. This finding along with reports that flavonoids can induce apoptosis in immortalized cancer cell lines [12–15], such as Jurkat T-lymphocytes, prompted us to determine if apoptosis occurred in Jurkat T-lymphocytes upon short-term incubation with either NDGA or curcumin. Accordingly, SDS-PAGE/Western-immunoblotting analysis was performed to detect any proteolytic cleavage of PARP. By doing so, we wished to eliminate the possibility that the comets appearing in curcumin-treated cells were due to the DNA fragmentation associated with apoptosis. Under our experimental conditions, PARP proteolysis did not occur in the curcumin-treated cells (Fig. 4A). Fully intact PARP (113 kD band), and not the characteristic proteolytic fragment (85 kD band) resulting from apoptosis, was found in cells treated for 30 min with curcumin concentrations as high as 100 μM. PARP proteolysis occurred only when cells were incubated with curcumin for a much longer time, i.e. 20h (Fig. 4B). Therefore, when incubated on a short-term basis with Jurkat T-lymphocytes, curcumin seems to exert its adverse effect on DNA without an immediate involvement of apoptosis.

To provide some insight on a possible mechanism by which curcumin damages DNA, an experiment was performed to determine if free radicals might be involved. As shown in Table 1, the possibility that H_2O_2 might be generated extracellularly was examined, which was prompted by a recent report [6]. Incubating RPMI-1640 culture medium with cur- cumin at concentrations up to 100 μM did not generate substantial levels of H_2O_2, in contrast to NDGA and epigallocatechin gallate. In other experiments, Jurkat T-lymphocytes were first fortified with the

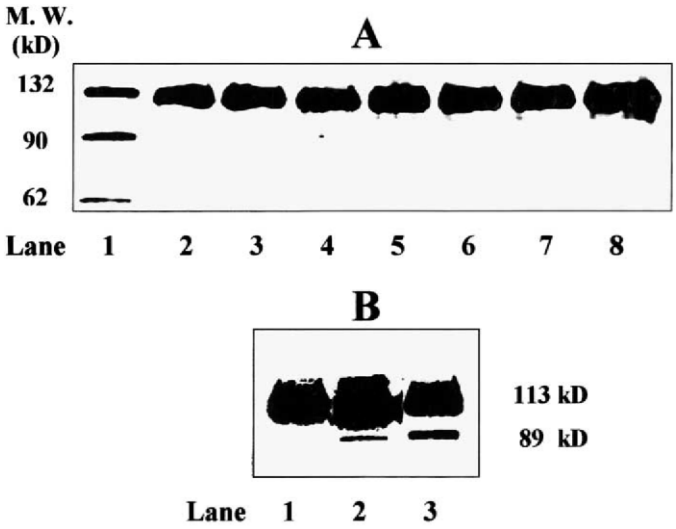


Fig. 4. Detection of an early marker of apoptosis, i.e. proteolytic cleavage of poly-(ADP ribose) polymerase or PARP (MW — 113 kD), in Jurkat T-lymphocytes upon short- and long-term incubation with curcumin. In panel A, cells were incubated with curcumin for 30 min. Then, whole cell extracts were prepared and subjected to SDS-PAGE/Western-immunoblotting. Samples are as follows: lane 1: molecular weight markers; lane 2: 0 μM curcumin; lane 3: 1 μM curcumin, lane 4: 5 μM curcumin; lane 5: 10 μM curcumin; lane 6: 25 μM curcumin; lane 7: 50 μM curcumin; and lane 8: 100 μM curcumin. Lack of an 85 kD band in any of the lanes indicates that PARP cleavage did not occur. In panel B, cells were incubated with 0 (lane 1), 50 (lane 2) and 100 (lane 3) μM curcumin for 20 h for subsequent SDS-PAGE/Western-immunoblotting. Presence of an 85 kD band in lanes 2 and 3 indicates that PARP cleavage occurred.

Table 1
Concentrations of hydrogen peroxide equivalents in cell culture medium after being incubated with different concentrations of phenolic phytochemicals[a]

Test substance	Concentration of H_2O_2 equivalents in medium (μM)
Control	None detected
Curcumin (μM)	
5	2.8 ± 0.8
25	2.2 ± 0.5
50	5.2 ± 3.0
100	2.7 ± 1.2
Nordihydroguaiaretic acid (μM)	
5	9.0 ± 1.3
25	12.0 ± 2.4
50	19.0 ± 1.6
100	32.2 ± 0.9
Epigallocatechin gallate (μM)	
5	4.2 ± 1.2
25	12.5 ± 0.3
50	32.0 ± 0.9
100	79.5 ± 4.6

[a] RPMI-1640 was incubated with the phenolic phytochemicals for 30 min and then H_2O_2 determined as described in Section 2. Values are the mean ± S.E.M. from four different experiments.

lipophilic antioxidant, α-tocopherol, by incubating the cells with 100μM α-tocopherol for 24h. Such incubation conditions resulted in about a seven-fold increase in α-tocopherol concentrations in HEL erythroleukemia cells [16] that is somewhat similar to Jurkat T-lymphocytes. The cells fortified with α-tocopherol were then treated with 5 μM curcumin to induce DNA damage. The comet assay of the cells are shown in Fig. 5. Control cells had undamaged DNA, as indicated by the absence of any distinct comets (panel A, comet score: 2 ± 1). Treatment of the cells with curcumin resulted in DNA damage, as indicated by the presence of comets (panel B, comet score: 94 ± 13). However, fortification of the cells with α-tocopherol prevented the curcumin-induced DNA damage (panel C, comet score: 11 ± 8).

4. DISCUSSION

The antioxidant properties of phenolic phytochemicals have received much attention [1,2,5]. However, phenolic phytochemicals can have both antioxidant

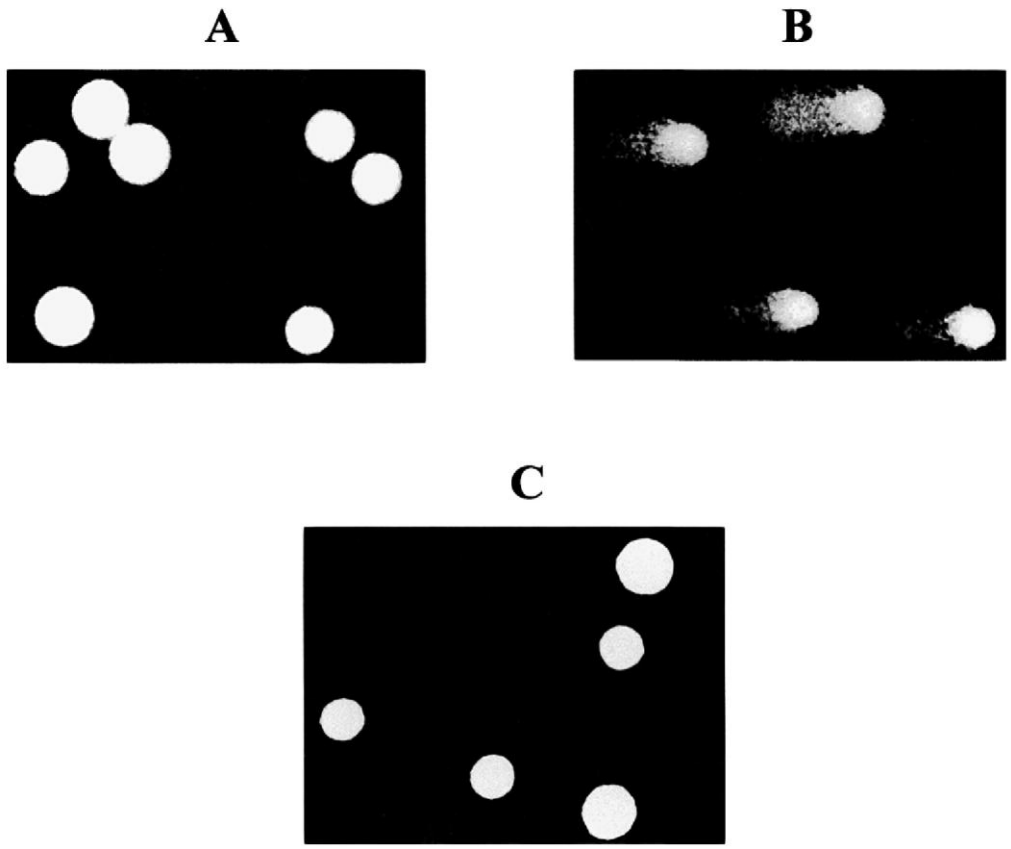


Fig. 5. Inhibition of curcumin-induced DNA damage in Jurkat T-lymphocytes by α-tocopherol, as evaluated by the comet assay. Cells in complete media were pre-incubated with 100 μM α-tocopherol for 24 h in a CO_2 incubator at 37°C. The cells were then washed and resuspended in phosphate-buffered saline, before being incubated with 5 μM curcumin for 30 min on ice. The comet assay was then performed. Panel A: control; panel B: 5 μM curcumin; panel C: 100 μM α-tocopherol/5 μM curcumin.

and prooxidant activities [17]. The general focus of the present study was to evaluate whether the structurally similar phenolic phytochemicals, NDGA and curcumin, would exert similar cellular effects in terms of protecting DNA against oxidative damage.

In the case of NDGA, its antioxidant activity was obvious based on results of the comet assay. In a concentration-dependent manner, NDGA inhibited DNA damage in Jurkat T-lymphocytes oxidatively challenged with H_2O_2. Similar results have been obtained with other phenolic phytochemicals. For example, it was demonstrated recently that quercetin and myricetin can inhibit H_2O_2-induced DNA damage in human peripheral blood lymphocytes [18], as evaluated by the comet assay. Phenolic phytochemicals in green tea inhibited H_2O_2-induced DNA damage in cultured human bronchial cells [19], but as evaluated by the DNA microfiltration assay. Mechanistically, NDGA likely protected the DNA in Jurkat T-lymphocytes lymphocytes by providing hydrogen atoms from its four phenolic hydroxyl groups to react with hydroxyl radicals, which are formed when the added H_2O_2 reacts with trace ferrous ions (Fenton reaction) [20]. The hydroxyl radicals attack the deoxyribose moeity of DNA to cause the strand breaks [21] that were detected by the comet assay. Upon releasing the hydrogen atoms, it is also possible that the resulting phenolate anion structure of NGDA chelated ferrous ions [5] to prevent the Fenton reaction from occurring to generate hydroxyl radicals.

In studying curcumin, contradictory results have been obtained. For example, the antioxidant properties of curcumin are well established. In simple test tube experiments with the purified compound, curcumin scavenged

superoxide anion and hydroxyl radicals, and also inhibited lipid peroxidation [22]. In cellular experiments, curcumin suppressed the generation of reactive oxygen species in stimulated macrophages [22,23]. On the other hand, curcumin can apparently act as a prooxidant. Our experiments suggest that short-term incubation of cells with curcumin can paradoxically result in the generation of reactive oxygen species that damage DNA as detected by the comet assay. It is noteworthy that fortification of cells with α-tocopherol, a potent neutralizer of reactive oxygen species [24], prevented the curcumin-induced DNA damage.

The ability of curcumin to induce DNA damage under certain experimental conditions has been demonstrated recently by others but using purified DNA [25]. When either calf thymus or plasmid DNA was incubated with curcumin along with cupric chloride, DNA strand breakage occurred that was attributed to reactive oxygen species. It was shown that curcumin directly generates superoxide anion, hydrogen peroxide, and in the presence of copper ions, hydroxyl radicals. These findings are consistent with the probability that the DNA damage induced by H2O2 or curcumin in Jurkat-T-lymphocytes may be ultimately due to hydroxyl radicals. It is known that hydroxyl radicals can be formed by reaction of H2O2 with metal ions such as copper or iron [20], which are present in trace amounts in cells.

Other phenolic phytochemicals can also damage DNA. Morin and naringenin each caused lipid peroxidation in isolated rat liver nuclei that was accompanied by DNA damage [26]. In the presence of added copper ions, tea catechins generated reactive oxygen species that led to DNA damage [27]. Quercetin induced DNA damage in HepG2 and human lymphocytes, although myricetin and silymarin did not [28]. In the present study, NDGA did not induce DNA damage. Instead, NDGA inhibited DNA damage caused by H2O2. Therefore, not all members of the phenolic phytochemical family exert antioxidant effects in terms of protecting cellular DNA against oxidative damage. Apparently, some phenolic phytochemicals can cause DNA damage in a process involving free radicals.

Recently, it was reported [6] that adding certain phenolic phytochemicals to cell culture media (cell-free) generates H_2O_2, and it was suggested that as an artifact this could contribute to the cellular effects of the phenolic phytochemicals. In our study, we did not find any appreciable generation of H_2O_2 after incubating the complete culture medium for Jurkat T-lymphocytes with curcumin. Therefore, it appears that if H_2O_2 is the indirect DNA-damaging agent in curcumin-treated Jurkat T-lymphocytes, the H_2O_2 is generated intracellularly after uptake of the curcumin. This possibility remains to be investigated, although it cannot be ruled out that other reactive chemical species may be responsible for curcumin-induced DNA damage as well.

Curcumin has been reported to induce apoptosis in a number of immortalized cancer cell lines [29,30]. But, such cells were incubated with curcumin for 16–48 h, which are incubation times far exceeding the 0.5 h used in the present study. Nevertheless, it is conceivable that the comets indicative of single strand breaks in DNA reflects an apoptotic event in our Jurkat T-lymphocytes treated with curcumin. During apoptosis, DNA is typically fragmented by a caspase-activated DNase [31]. Such DNA fragmentation can appear as comets upon comet assay of apoptotic cells [10]. However, PARP proteolysis, a definitive early marker of apoptosis [9], was not detected in cells incubated with curcumin for 0.5h. Therefore, short incubation of Jurkat T-lymphocytes with curcumin did not induce apoptosis. In other words, the curcumin-induced damage to DNA visible as comets cannot be due to DNase-mediated fragmentation of DNA, a rather late event in apoptosis. Upon long-term incubation of cells with curcumin, it is reasonable to think that the initial damage to DNA induced by curcumin and unable to be successfully repaired by nucleotide excision repair reactions [32] is followed by initiation of apoptosis. Hence, PARP proteolysis and then DNA fragmentation would subsequently become evident.

Our results imply that the mechanism of action for the known anti-tumor effect of curcumin [4] is different from the mechanism of action for the known anti-tumor effect of NDGA [3]. That is, curcumin might prevent tumorigenesis by inducing apoptosis in newly emerging cancer cells. It might do so by promoting the formation of reactive oxygen species, which then cause irreparable oxidative damage of cancer cell DNA and consequent initiation of apoptosis [33]. In contrast, NDGA might prevent tumorigenesis by simply neutralizing free radicals

that can damage the DNA of normal cells. Hence, mutation of the genome and ensuing transformation of normal cells to cancerous cells are prevented. On the other hand, cell culture experiments [34] have shown that long-term incubation (24h) of various types of cancer cell lines with NDGA also induces apoptosis. In association with the apoptosis, NDGA promoted lipid peroxidation. NDGA-induced apoptosis was inhibited in cells pre-treated with antioxidants. Therefore, under experimental conditions different from what we used, it is possible that NDGA might induce apoptosis in our Jurkat T-lymphocytes.

In summary, under our experimental conditions, the two phenolic phytochemicals we studied had dissimilar effects. NDGA prevented DNA damage in Jurkat T-lymphocytes oxidatively stressed with hydrogen peroxide, whereas curcumin did not to any extent whatsoever. Instead, curcumin caused highly discernible DNA damage in these cells. Curcumin seems to damage DNA through free radicals since ot-tocopherol prevented curcumin-induced DNA damage.

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