EFFECT OF COPPER DEFICIENCY ON OXIDATIVE DNA DAMAGE IN JURKAT T-LYMPHOCYTES

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Abstract:
The micronutrient copper is a catalytic cofactor for copper, zinc superoxide dismutase and ceruloplasmin, which are two important antioxidant enzymes. As such, a lack of copper may promote oxidative stress and damage. The purpose of this study was to determine the effect of copper deficiency on oxidative damage to DNA in Jurkat T-lymphocytes. To induce copper deficiency, cells were incubated for 48 h with 5-20 μM 2,3,2-tetraamine (2,3,2-tet), a high affinity copper chelator. Such treatment did not affect cell proliferation/viability, as assessed by measuring mitochondrial reduction of WST-1 reagent (4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate). Furthermore, the induction of copper deficiency did not promote oxidative DNA damage as evaluated by the comet assay. Comet scores were 15 ± 0 and 16 ± 1 for control and copper-deficient cells, respectively. However, the copper-deficient cells sustained greater oxidative DNA damage than the control cells (comet scores of 175 ± 15 and 50 ± 10, respectively) when both were oxidatively challenged with 50 μM hydrogen peroxide (H_2O_2), Supplemental copper but not zinc or iron prevented the potentiation of the H_2O_2-induced oxidative DNA damage caused by 2,3,2-tet. These data suggest that copper deficiency compromises the antioxidant defense system of cells, thereby increasing their susceptibility to oxidative DNA damage.

Keywords:
Copper, DNA damage, Jurkat T-lymphocytes, Reactive oxygen species, Free radicals

Article:

INTRODUCTION

Antioxidants protect against excess reactive oxygen species (ROS) that can damage lipid, protein, and DNA. Regarding their general mechanism [1], antioxidants release hydrogen atoms to scavenge ROS or chelate metal ions that can facilitate formation of ROS [2]. Several essential trace elements, including copper [3], are atypical antioxidants because they work indirectly. Copper is a catalytic cofactor for copper,zinc superoxide dismutase (Cu,Zn SOD) and ceruloplasmin (Cp). Cu,Zn SOD catalyzes dismutation of superoxide anion, producing molecular oxygen and hydrogen peroxide (H_2O_2) with the latter product usually disposed of by glutathione peroxidase and catalase. The ferroxidase activity of Cp mediates the oxidation of ferrous ions to the ferric state, thereby preventing ferrous ion-dependent formation of hydroxyl radical via the Fenton reaction [4]. Thus, in enabling Cu,Zn SOD and Cp to function as described, copper can be classified as a part of the antioxidant defense system of cells.

The impact of copper deficiency on the antioxidant defense system and oxidative damage to cellular components has been a topic of considerable interest [3]. The activities of SOD, catalase, and glutathione peroxidase are decreased in liver and other tissues of rats fed a copper-deficient diet [5,6]. This response to an inadequate supply of copper may promote the oxidation of cellular proteins. Lower Cu,Zn SOD activity and higher

oxidative damage to membrane proteins of erythrocytes have been reported during copper deficiency [7]. Recently, it was found that depriving cultured HL-60 cells of copper increased levels of protein carbonyls in mitochondria [8], indicative of oxidative damage to proteins by ROS. Copper deficiency also increases peroxidation of membrane lipids. Greater production of breath ethane [9] and higher levels of thiobarbituric acid–reactive substances in liver [10] and heart [11] have been reported in copper-deficient animals. Collectively, these studies indicate that copper deficiency weakens the antioxidant defense system. Consequently, levels of ROS increase so that oxidative damage to lipids and proteins is enhanced.

In contrast, the effect of copper deficiency on oxidative DNA damage has received limited attention. This situation is likely due in part to the general view that excess, rather than inadequate, copper or other essential transition metals promotes oxidative DNA damage [2]. A recent study [12] suggested that copper deficiency promotes oxidative DNA damage. More specifically, copper-deficient rats had higher hepatic activity of nuclear DNA repair enzymes than in controls after treatment with aflatoxin B, suggesting greater oxidative damage to DNA as a result of copper deficiency. Interestingly, basal activities of the DNA repair enzymes were similar in the control and copper-deficient animals. These observations suggest that copper deficiency itself does not induce DNA damage, but enhances the susceptibility of DNA to oxidative damage. To examine these two possibilities in a cellular model, we first induced copper deficiency in Jurkat T-lymphocytes using the high affinity copper chelator, 2,3,2-tetraamine (2,3,2-tet) [13]. We then evaluated the extent of DNA damage in the cells before and after oxidative challenge with an exogenous ROS.

MATERIALS AND METHODS

Materials

Jurkat T-lymphocytes were obtained from ATCC (Rockville, MD, USA). Cell culture media and supplements, low and normal melting point agarose, and common reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The copper chelator reagent 2,3,2-tet (i.e., N,N'-Bis(2-aminoethyl)-1,3- propanediamine) was obtained originally from Eastman Chemical Co. (Rochester, NY, USA). The WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) cell proliferation assay kit was obtained from Boehringer Mannheim (Indianapolis, IN, USA).

Cell culture and induction of copper deficiency

Jurkat T-lymphocytes were grown in RPMI-1640 media supplemented with 6% fetal bovine serum, 2 mM glutamine, 0.54 µM fungizone, and antibiotics (100,000 U/L penicillin, 100 mg/L streptomycin) inside a humidified incubator (95% air/5% CO_2) set at 37°C. They were maintained at 1–20 X 10^6 cells/ml in T-75 flasks.

The copper status of Jurkat T-lymphocytes was decreased with 2,3,2-tet as reported elsewhere [14]. Briefly, cells were seeded into either 96 well microplates (2.5 X 10^4 cells/0.1 ml) or 12 well microplates (2.5 X 10^5 cells/1 ml) in complete medium with either 0, 5, 10, or 20 µM 2,3,2-tet, depending upon the subsequent assay. The cultures were then incubated for 48 h in the humidified incubator. To determine if the effects of 2,3,2-tet were due to copper deficiency, 25 µM of either cupric chloride, zinc chloride, or ferrous chloride were added to the cells at the same time that the 2,3,2-tet was added. After incubation, the cell samples were processed as described below.

Evaluation of cell proliferation/viability

Proliferation/viability of cell samples in the 96 well microplates were evaluated with the WST-1 cell proliferation assay kit following the instructions of the manufacturer. The results, which reflect the capacity of nicotinamide adenine dinucleotide (reduced form) (NADH)-dependent mitochondrial dehydrogenases to reductively cleave WST-1 reagent, were expressed as the percent of control.

Oxidative challenge of the cells with H2O2

Cell samples in the 12 well microplates were washed and resuspended in 10 mM phosphate-buffered saline (PBS), pH 7.4 . They were then incubated with 50 µM H_2O_2 for 15 min on ice. After terminating the incubation by washing with cold PBS, oxidative DNA damage was assessed.

Evaluation of oxidative DNA damage in the cells

Single-cell gel electrophoresis or comet assay was performed under alkaline conditions [15]. Briefly, the cells resuspended in 0.1 ml PBS were mixed with an equal volume of 1% low melting point agarose, and 75 µl of the mixture were pipetted onto a frosted glass microscope slide that had been precoated with 85 µl of 1% normal melting point agarose. Without delay, a coverslip was placed on the slide. Next, the slide was placed on an ice-cold metal tray for 10 min to allow the agarose layer to congeal. After removing the coverslips, all slides were immersed in a coplin jar containing cell lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 300 mM NaOH, 10% DMSO, and 1% Triton X-100, pH 10) at 4°C for 1 h. Afterwards, slides were placed in a horizontal electrophoresis unit containing freshly prepared electrophoresis buffer (300 mM NaOH/1 mM EDTA, pH 13). The DNA was allowed to unwind for 40 min before electrophoresis was performed at 20 V (1 V/cm, 300 mA) for 20 min. Afterwards, the slides were gently immersed in neutralization buffer (0.4 M Tris-HCl, pH 7.5) for 5 min, and this step repeated. Finally, 60 µl ethidium bromide solution (2 µg/ml) was pipetted on the slides followed by coverslips to stain the DNA.

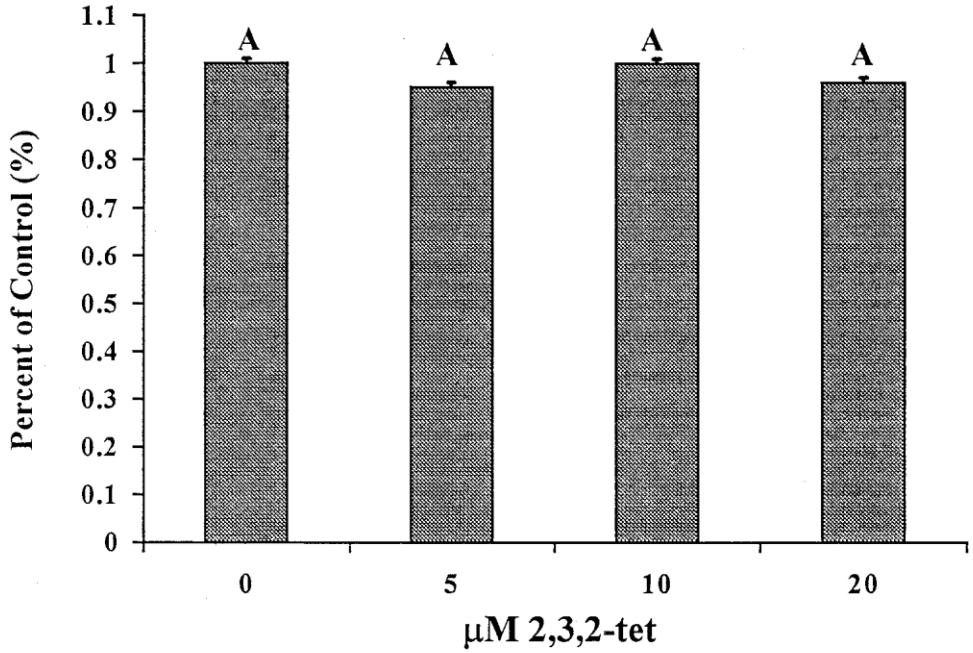

Fig. 1. Negative effect of 2,3,2-tetraamine (2,3,2-tet) on viability and general metabolic activity of Jurkat T-lymphocytes. Cells (2.5×10^4/0.1 ml media) were seeded into 96 well microplates and incubated with 0–20 μM 2,3,2-tet for 48 h. WST-1 reagent (0.01 ml) was added to each well and further incubated for 2 h. Absorbance of the cell supernate was measured at 450 nm. Values are the mean ± standard deviation from five determinations. Values sharing the same letter are not significantly different from one another ($p < .05$).

Using the standard rhodamine filter, slides were viewed on an Olympus IX-70 inverted fluorescence microscope equipped with a PM-C35DX camera. Fifty nucleoids were visually scored for comets on a scale of 0–4, as described [16]. A nucleoid appearing essentially as a comet "head" was scored 0, indicating no or minimum DNA damage. A nucleoid exhibiting a comet "tail" was scored either 1, 2, 3, or 4 depending on the overall span of the tail. Despite being subjective, this scoring system compares acceptably with computer imaging analysis [17].

Statistical analysis of data

Data were analyzed for significant differences ($p < .05$) by analysis of variance (ANOVA) and Duncan's New Multiple Range Test using a SAS (Cary, NC, USA) program.

RESULTS

To determine if treatment of Jurkat T-lymphocytes with 2,3,2-tet for 48 h was cytotoxic, the WST-1 cell proliferation/viability assay was performed. As shown in Fig. 1, 2,3,2-tet did not decrease the ability of the cells to reductively cleave WST-1 reagent (a tetrazolium salt) via NADH-dependent mitochondrial dehydrogenases. At 2,3,2-tet concentrations of 0, 5, 10, or 20 μM, the measured absorbances reflecting reduction of WST-1 reagent were not different ($p < .05$) among the cell groups. Therefore, cell proliferation/viability was not reduced by 2,3,2-tet or the copper deficiency caused by it, consistent with a previous study [14].

To determine the extent of cellular DNA damage, the comet assay was performed. As seen in Figs. 2 and 3, control cells showed nucleoids with minimal comet formation and had a comet score of 15 ± 0. Essentially all of the DNA appeared within the perimeter of the nucleoid mass, indicating no significant extent of DNA single-strand breaks due to oxidative damage. A similar nucleoid profile was also observed in 2,3,2-tet–treated cells, which had a comet score of 16 ± 1. Therefore, the copper deficiency induced by 2,3,2-tet in Jurkat T-lymphocytes by itself did not promote oxidative DNA damage.

When control and 2,3,2-tet–treated cells were subsequently incubated with H_2O_2, differences in susceptibility of the cell samples to H_2O_2-induced oxidative DNA damage were apparent (Figs. 2 and 4). As expected, control cells challenged with H_2O_2 showed nucleoids resembling comets and had a comet score of 50 ± 10. While most of the DNA appeared within the comet "heads," a noticeable amount of DNA appeared within the comet "tails." The latter visual feature is indicative of substantial single strand breaks in DNA, and hence,

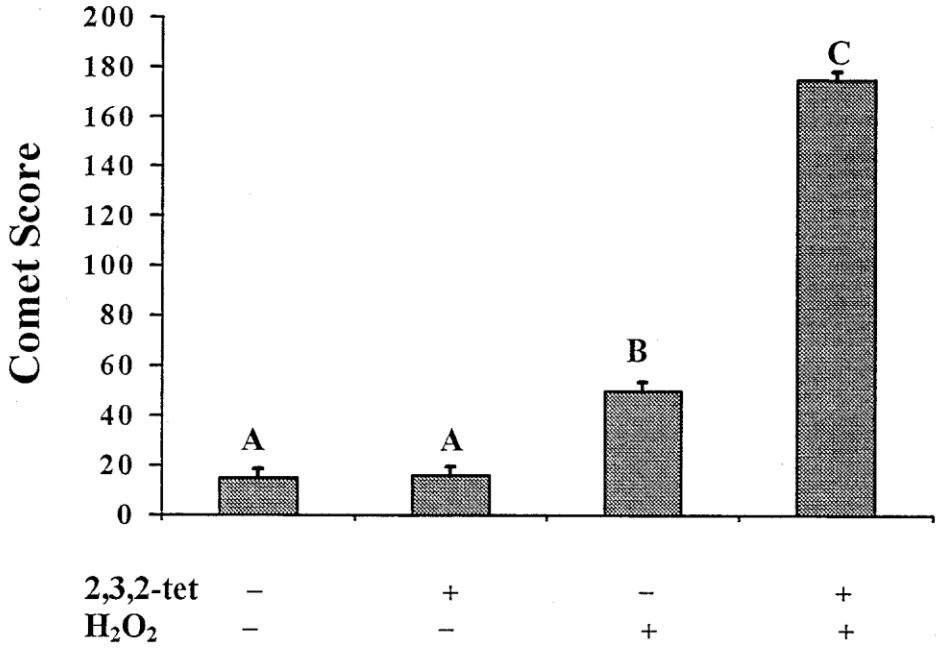

Fig. 2. Effect of copper deficiency on DNA damage in Jurkat T-lymphocytes. Cells were incubated for 48 h with and without 20 μM 2,3,2-tetraamine (2,3,2-tet). Afterwards, cells were incubated with and without 50 μM hydrogen peroxide (H_2O_2) for 15 min. The comet assay was performed as described under Materials and Methods. Values are the mean ± SD of data from three separate experiments. Values not sharing the same letter are significantly different from one another ($p < .05$).

oxidative damage to DNA. In contrast, 2,3,2-tet–treated cells had more extreme comets (comet score: 175 ± 15) when challenged with H_2O_2. That is, no comet heads were visible, and essentially all the DNA appeared within the comet tails. The presence of such comets reflects massive destruction of DNA. Therefore, the copper deficiency induced by 2,3,2-tet in Jurkat T-lymphocytes increased their susceptibility to oxidative DNA damage.

It was important to determine if copper, but not other essential trace metals, could prevent the potentiation of H_2O_2-induced oxidative DNA damage caused by 2,3,2-

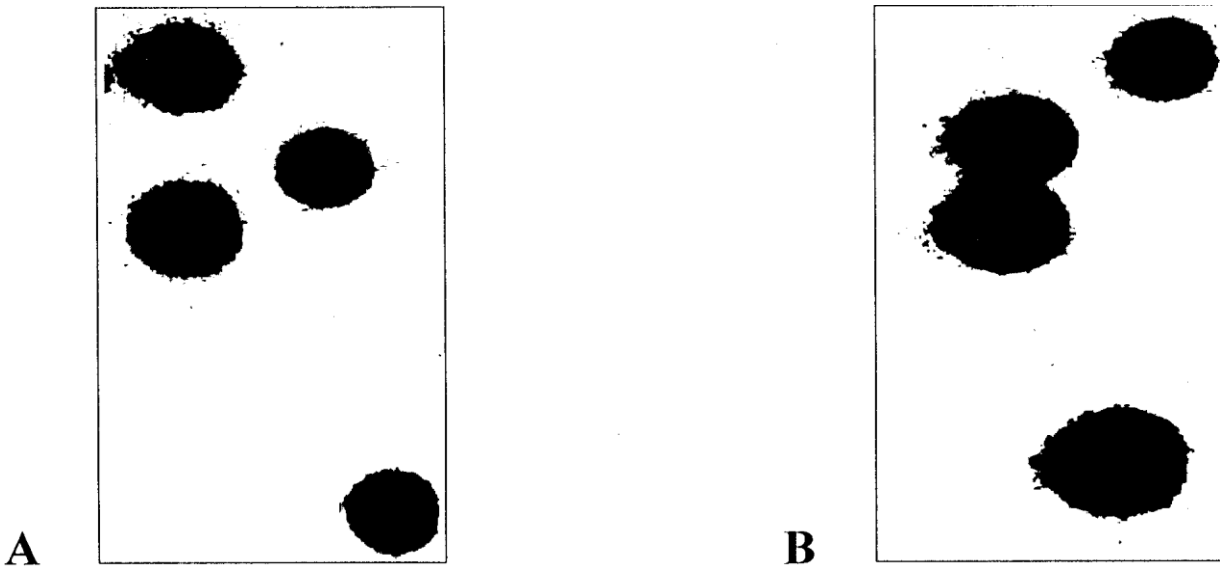

Fig. 3. Negative effect of copper deficiency alone on DNA damage in Jurkat T-lymphocytes. The scanned images are representative of the comet assay of control cells (A) and 2,3,2-tetraamine-treated cells (B).

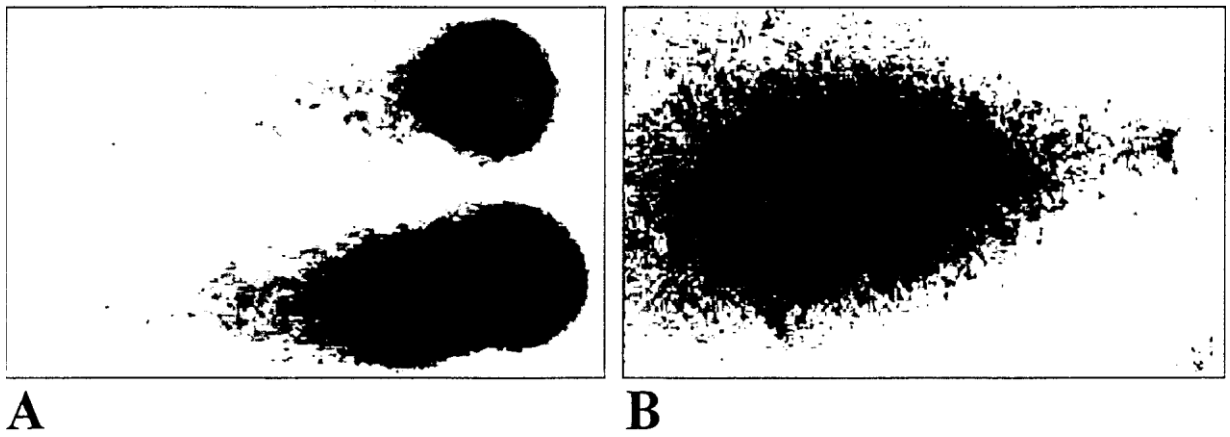

Fig. 4. Enhancement by copper deficiency of the susceptibility of Jurkat T-lymphocytes to DNA damage induced by hydrogen peroxide (H_2O_2). The scanned images are representative of the comet assay of control cells (A) and 2,3,2-tetraamine-treated cells (B) after both were incubated with H_2O_2 for 15 min.

tet (Fig. 5). Jurkat T-lymphocytes were simultaneously treated with 20 µM 2,3,2-tet and 25 µM cupric chloride. The cells were then challenged with H_2O_2 and the comet assay performed. Consistent with our previous experiment, 2,3,2-tet–treated cells subsequently challenged with H_2O_2 sustained greater DNA damage (comet score: 185 ± 14) than control cells challenged with H_2O_2
(comet score: 50 ± 14). However, cells exposed to 2,3,2-tet and supplemental copper and then subsequently challenged with H_2O_2 had the same comet score (50 ± 14) as the control cells challenged with H_2O_2. In contrast, cells exposed to 2,3,2-tet and supplemental zinc or iron then subsequently challenged with H_2O_2 had comet scores of 170 ± 28 and 171± 27, respectively. There-

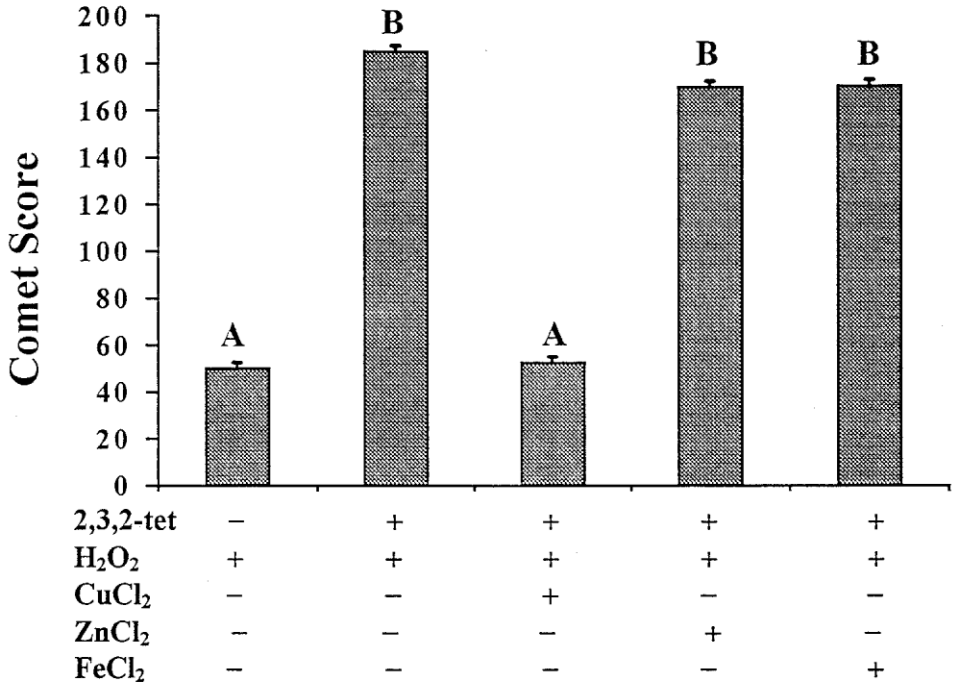

Fig. 5. Prevention by copper, but not zinc or iron, of hydrogen peroxide (H_2O_2)-induced DNA damage in Jurkat T-lymphocytes exposed to 2,3,2-tetraamine (2,3,2-tet). Cells were incubated for 48 h with 20 μM 2,3,2-tet and 25 μM of either cupric chloride ($CuCl_2$), zinc chloride ($ZnCl_2$), or ferrous chloride ($FeCl_2$). After challenging the cells with 50 μM H_2O_2 for 15 min, the comet assay was performed as described under Materials and Methods. Values are the mean ± SD of data from two separate experiments. Values not sharing the same letter are significantly different from one another ($p < .05$).

fore, only copper prevented the potentiation of H_2O_2- induced oxidative DNA damage caused by 2,3,2-tet.

DISCUSSION

The micronutrient copper plays an important supporting role in scavenging excess ROS and preventing oxidative damage to critical cellular components. It is often assumed that failure to provide cells and tissues with an adequate supply of copper increases their susceptibility to oxidative stress. Indeed, previous studies have established that copper deficiency increases oxidative damage to lipids [10,11] and proteins [7,8]. To our knowledge, this study is the first one to examine the effect of copper deficiency on oxidative DNA damage in a cultured cell line.

Copper deficiency can be induced in cultured cells by different means. One way is to subculture cells in copper-deficient media for several passages. Using this approach, it was recently reported that copper deficiency induced oxidative stress in HL-60 human leukemic cells [8]. Higher levels of protein carbonyl groups were detected in mitochondria isolated from the copper-deficient HL-60 cells, suggesting the presence of elevated levels of ROS in such cells. Copper deficiency was induced in Jurkat T-lymphocytes by simply incubating the cells with 2,3,2-tet to partially deplete them of copper. This chelator was previously shown to effectively decrease copper content and Cu,Zn SOD activity in Jurkat cells [14]. Hence, it is likely that 2,3,2-tet had similar effects in Jurkat cells used in the present study. Moreover, cell viability and general metabolic activity were not compromised, as was substantiated with the WST-1 cell proliferation/viability assay in the present study.

Although we did not directly measure levels of ROS in 2,3,2-tet–treated cells, any oxidative stress caused by the partial copper depletion was apparently not sufficient to damage DNA, as evaluated by the sensitive comet assay. There was no difference in net comet scores between control and copper-deficient Jurkat T-lymphocytes. Thus, this finding indicates that the antioxidant defense system, which is comprised of primary and secondary antioxidants as well as antioxidant enzymes, was not sufficiently compromised to result in oxidative DNA damage in the absence of exogenous stressor. However, copper deficiency decreased the ability of the cells to

resist oxidative damage to DNA when challenged with H_2O_2. This observation is similar to the situation where the oxidative stress of vitamin E deficiency [18] may be potentiated by that of cigarette smoking [19].

In attempting to explain the enhanced susceptibility of copper-deficient Jurkat T-lymphocytes to oxidative DNA damage, copper's role as an indirect antioxidant seems apparent [3]. Thus, it is reasonable to speculate that lack of copper in Jurkat T-lymphocytes reduces the activities or concentrations of components of the antioxidant defense system, thereby facilitating oxidative DNA damage. It was reported previously that treating Jurkat T-lymphocytes with 2,3,2-tet resulted in 30–40% reduction of Cu,Zn SOD activity [14]. Animal studies also have shown that copper deficiency is associated with decreased activities of the noncopper-containing enzymes, glutathione peroxidase [5,20,21], and catalase [11,22]. Similar declines in the activity of these enzymes in copper-deficient Jurkat T-lymphocytes would increase susceptibility to H_2O_2-induced oxidative DNA damage. That is, the decreased enzyme activity would lead to a higher concentration of H_2O_2 when added to the copper-deficient cells, therefore allowing a greater load of H_2O_2 to diffuse into the nucleus and react with metal ions to form hydroxyl radicals that ultimately damage DNA. Obviously, additional studies are needed to elucidate why copper deficiency enhances oxidative DNA damage in Jurkat cells and to examine if the response to the micronutrient deficiency reflects a general response to inadequate copper availability.

In conclusion, it is evident that normal levels of certain nutrients are needed to prevent oxidative damage to cellular components. Excess or deficient levels of trace metals may have adverse effects on DNA in particular. For example, the presence of excess metal ions can initiate the formation of hydroxyl radical [2] that will damage DNA [23]. However, as the current study shows, metal ion deficiency also may promote oxidative DNA damage. Thus, normal levels of copper must be present in cells to maintain the structural integrity of DNA during oxidative stress.

REFERENCES

[1] Shahidi, F,; Wanasundara, P, K, J, P, D, Phenolic antioxidants, Crit. Rev. Food Sci. Nutr. 32:67–103; 1992,

[2] Halliwell, B,; Gutteridge, J, M, C, Role of free radicals and catalytic metal ions in human disease: an overview, Methods Enzymol. 186:1–85; 1990,

[3] Johnson, M, A,; Fischer, J, G,; Kays, S, E, Is copper an antioxidant nutrient? Crit. Rev. Food Sci. Nutr. 32:1–31; 1992,

[4] Gutteridge, J, M, Inhibition of the Fenton reaction by the protein ceruloplasmin and other copper complexes, Assessment of ferroxidase and radical scavenging activities, Chem. Biol. Inter. 56:113–120;1985,

[5] Prohaska, J, R, Changes in Cu,Zn-superoxide dismutase, cytochrome c oxidase, glutathione peroxidase, and glutathione transferase activities in copper-deficient mice and rats, J. Nutr. 121: 355–363; 1991,

[6] DiSilvestro, R, A,; Medeiros, D, M, Low and marginal copper intake by postweanling rats: effects on copper status and resistance to carbon tetrachloride hepatotoxicity, Metabolism 41:1122–1124;1992,

[7] Sukalski, K, A,; LaBerge, T, P,; Johnson, W, T, In vivo oxidative modification of erythrocyte membrane proteins in copper deficiency, Free Radic. Biol. Med. 22:835–842; 1997,

[8] Johnson, W, T,; Thomas, A, C, Copper deprivation potentiates oxidative stress in HL-60 cell mitochondria, Proc. Soc. Exp. Biol. Med. 221:147–152; 1999,

[9] Saari, J, T,; Dickerson, F, D,; Habib, M, P, Ethane production in copper-deficient rats, Proc. Soc. Exp. Biol. Med. 195:30–33; 1990,

[10] Rayssiguier, Y,; Gueux, E,; Bussiere, L,; Mazur, A, Copper deficiency increases the susceptibility of lipoproteins and tissues to peroxidation in rats, J. Nutr. 123:1343–1348; 1993,

[11] Chen, Y,; Saari, J, T,; Kang, Y, J, Weak antioxidant defenses make the heart a target for damage in copper-deficient rats, Free Radic. Biol. Med. 17:529–536; 1994,

[12] Webster, R, P,; Gawde, M, D,; Bhattacharya, R, K, Modulation by dietary copper of aflatoxin B1–induced activity of DNA repair enzymes poly (ADP-ribose) polymerase, DNA polymerase beta, and DNA ligase, In Vivo 10:533–536; 1996,

[13] Fawcett, T, G,; Rudich, S, M,; Toby, B, H,; Lalancette, R. A,; Potenza, J, A,; Schugar, H, J, Studies of chelation therapy, Crystal and molecular structure of Cu[H2N(CH2)2NH(CH2)3-NH(CH2)2NH2](ClO4)2, Cu(2,3,2-tet) (ClO4)2, Inorg. Chem. 19:940–945; 1980,

[14] Hopkins, R, G,; Failla, M, L, Copper deficiency reduces interleukin-2 (IL-2) production and IL-2 mRNA in human T-lymphocytes, J. Nutr. 127:257–262; 1997,

[15] Singh, N, P,; McCoy, M, T,; Tice, R, R,; Schneider, E, L, A simple technique for quantitation of low levels of DNA damge in individual cells, Exp. Cell Res. 175:184–191; 1988,

[16] Duthie, S, J,; Johnson, W,; Dobson, V, L, The effect of dietary flavonoids on DNA damage (strand breaks and oxidized pyrimidines) and growth in human cells, Mutation Res. 390:141–151; 1997,

[17] McCarthy, P, J,; Sweetman, S, F,; McKenna, P, G,; McKelvey- Martin, V, J, Evaluation of manual and image analysis quantitation of DNA damage in the alkaline comet assay, Mutagenesis 12:209–214; 1997,

[18] Mazor, D,; Brill, G,; Shorer, Z,; Moses, S,; Meyerstein, N, Oxidative damage in red blood cells of vitamin E– deficient patients, Clin. Chim. Acta 265:131–137; 1997,

[19] Nadiger, H, A,; Mathew, C, A,; Sadasivudu, B, Serum malondialdehyde (TBA reactive substance) levels in cigarette smokers, Atherosclerosis 64:71–73; 1987,

[20] Arthur, J, R,; Morrice, P, C,; Nicol, F,; Beddows, S, E,; Boyd, R,; Hayes, J, D,; Beckett, G, J, The effects of selenium and copper deficiencies on glutathione S-transferase and glutathione peroxidase in rat liver, Biochem J. 248:539–544; 1987,

[21] Olin, K, L,; Walter, R, M,; Keen, C, L, Copper deficiency affects selenoglutathione peroxidase and selenodeiodinase activities and antioxidant defense in weanling rats, Am. J. Clin. Nutr. 59:654– 658; 1994,

[22] Taylor, C, G,; Bettger, W, J,; Bray, T, M, Effect of dietary zinc and copper deficiency on the primary free radical defense system in rats, J. Nutr. 118:613–621; 1988,

[23] Breen, A, P,; Murphy, J, A, Reactions of oxyl radicals with DNA, Free Radic. Biol. Med. 18:1033–1037; 1995,

ABBREVIATIONS

2,3,2-tet—2,3,2-tetraamine
H_2O_2—hydrogen peroxide
W S T-1 —(4- [3 -(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5- tetrazolio]-1,3-benzene disulfonate)
ROS—reactive oxygen species
Cu,Zn SOD—copper,zinc superoxide dismutase Cp—ceruloplasmin
PBS—phosphate-buffered saline
DMSO—dimethyl sulfoxide EDTA—ethylenediaminetetraacetic acid