— Research Letter —

Chloroacetaldehyde Induces Chromosome Aberrations and Micronucleus Formation but Not 2-chloroethanol

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2-Chloroethanol (2-CE) has been used on grapevines to accelerate grape growth, and its metabolite, chloroacetaldehyde (CAA), accumulated in the liver and blood from rats intoxicated with 2-CE. Chronic occupational injury might be a possible reason for the 2-CE intoxication. In this study, we used the *in vitro* and *in vivo* tests to examine the genotoxicity of 2-CE and CAA. First, 2-CE did not induce chromosome aberration formation in Chinese ovary hamster cells, but CAA did induce chromosome aberration formation, especially the chromosome gap-type aberration after S9 activation. Second, 2-CE at high doses (1/2 LD_{50}), but not at low doses, induced peripheral blood micronucleus formation in mice. CAA induced micronucleus formation at low and high dosages (1/8–1/2 LD_{50}). These results indicated that CAA plays an important role in 2-CE chronic intoxication, and the genotoxic mechanisms of CAA require further study.

Key words —— 2-chloroethanol, chloroacetaldehyde, genotoxicity, chromosome aberration, micronucleus

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INTRODUCTION

2-Chloroethanol (2-CE) has been used on grapevines to accelerate grape growth in Taiwan. Although it has been prohibited, fatal intoxication is still annually reported.[1)] Chloroacetaldehyde (CAA) is known to accumulate and can be detected in the liver and blood of rats intoxicated with 2-CE.[2,3)] CAA is a bi-functional agent produced by the rearrangement of chloroethylene oxide, the main metabolite of the human and animal carcinogen vinyl chloride.[4)] Mutagenicity studies demonstrated that CAA induced gene mutations in prokaryotic and eukaryotic cells.[5–9)] Also, etheno derivatives of nucleic acids were identified as reaction products of CAA with DNA.[10,11)] Increased frequencies of sister chromatid exchange (SCE) have been observed in workers who are occupationally exposed to vinyl chloride monomer (VCM).[12)] CAA is an effective inhibitor of DNA synthesis;[13)] it forms interstrand crosslinks with DNA *in vitro*,[14)] modifies DNA conformation,[15)] and reacts with nucleotide bases.[10,16)] To our knowledge, no information is available on the ability of CAA to affect chromosome structure and segregation or micronucleus formation.

MATERIALS AND METHODS

Chromosome Aberration (CA) Test —— The Chinese hamster ovary (CHO-K1) cell line used for the CA test was obtained from the Food Industry Research and Development Institute (FIRDI, Hsinchu, Taiwan). Cells were maintained in monolayer conditions in F-12 medium (GIBCO, Grand Island, NY, U.S.A.) supplemented with 10% foetal bovine serum (FBS, GIBCO), at 37°C in a 5% CO_2 atmosphere incubator. According to our cytotoxicity results, 100, 75, and 50% of LC_{50} were used as the dosages. The lethal concentration 50 (LC_{50}) of 2-CE was over 10 mM, and the CAA was 0.35 mM. For each treatment, cells were cultured in duplicate in a T-25 flask (Orange Scientific, Brussel, Belgium), and treated with the 2-CE or CAA for 24 hr. A metabolic activation treatment was then carried out with/without S9 mixture (Lot. 1452, Aroclor 1254-induced rat liver, Moltox™, Boone, NC, U.S.A.). Cyclophosphamide (10 μM, Sigma, St. Louis, MO, U.S.A.) was used as a positive control in the presence of S9 mixture, and ethyl methane sulfonate (EMS, 2.5 μM) was used as a

positive control in the absence of S9.[17] Colcemid was then added to each flask during the last 3 hr of incubation. Chromosome preparation was performed using standard methods,[18] and cells were incubated in a hypotonic KCl (75 mM) solution at 37°C for 30 min and fixed in methanol : acetic acid (3 : 1, v/v). Slides were prepared and two drops of fixed cell suspensions were spread on glass slides and air-dried. Cells were then stained with a 5% Giemsa solution, and the slides were dried. At least 60 metaphases were then counted at 1000 × under an optical microscope.

Micronucleus Assay —— Five-week-old male mice of the ICR strain were acclimatized for at least 7 d before being subjected to the micronucleus test. The treatment groups were as follows: vehicle alone (dimethyl sulfoxide, DMSO, negative control) and positive control group (cyclophosphamide 50 mg/kg dissolved in sterile deionized water). ICR mice were intraperitoneally (i.p.) injected with 10, 20, and 40 mg/kg of 2-CE, or with 0.75, 1.5, and 3 mg/kg of CAA. Doses used were obtained from previously described 1, 1/2, and 1/4 i.p. LD_{50} dosages.[19] Animals were administered i.p. to test the substances and were harvested for peripheral blood at 48 and 72 hr after treatment. The peripheral blood was spread on slides and air-dried. The slides were then fixed in methanol for 5 min and stained using a Diff-Quick stain technique. Slides were observed under an optical microscope at 1000 ×,[20] and numbers of micronucleated cells were determined by counting numbers of normochromatic erythrocytes (NEC) from at least 1000 NEC per animal. Micronucleated reticulocytes (MNRET) that contained micronuclei were counted from at least 1000 RET stained with acridine orange (AO).[21] The study protocol was approved by the animal research ethics committee at National Chung Hsing University, Taichung, Taiwan (IACUC Approval No: 98-57).

Statistical Analysis —— Data are expressed as the means ± standard deviations. Statistical differences were evaluated by Dunnett's test. Differences were regarded as significant at $p < 0.05$.

RESULTS AND DISCUSSION

CAA but Not 2-CE Induced Chromosome Aberration

Chromosome aberration including gap, breakage, and exchanges were induced by CAA at concentrations ranging from 0.263 to 0.35 mM, as compared to the untreated controls with S9 mix only (Table 1). 2-CE did not produce chromosome aberrations in CHO-K1 cells. Our results showed that 2-CE did not cause chromosome aberrations with or without S9 activation at the 10 μM (LC_{50}) concentration. Ivett *et al*. (1989) have shown that 2-CE induced chromosome aberrations;[22] however, in the trials without activation, significant increases were detected only at doses well above the limits of the protocol (5000 μg/ml). Our results indicated that CAA induced chromosome aberrations after S9 mix activation. The frequencies of chromosome aberration were 60.5% for chromosome gap, 20.1% for chromosome breakage, and 19.4% for chromosome exchange. Chiang *et al*. (1997) have shown that CAA displayed greater toxicity and a larger frequency of deletion mutations on human B-lymphoblastoid cell line.[23] Crebelli *et al*. (1990) also indicated that CAA induced mitotic aneuploidy in yeast.[9] In our results, after S9 activation, CAA, but not 2-CE, induced gap-type chromosome aberrations in CHO-K1 cells. This study is the first report of CAA-induced chromosomal aberrations.

Table 1. Chromosome Aberration Frequency for 2-CE and CAA

Treatment		Frequency of Aberrations (%)	
		−S9	+S9
Negative Control		11.9 ± 4.7	13.3 ± 2.9
2-CE (mM)	5	13.3 ± 4.4	15.5 ± 1.9
	7.5	12.5 ± 0.9	17.8 ± 2.5
	10	11.6 ± 1.7	17.2 ± 4.8
CAA (mM)	0.175	15.5 ± 2.6	12.7 ± 2.3
	0.263	13.2 ± 2.9	28.3 ± 2.9*
	0.35	17.7 ± 3.4	41.6 ± 3.4*
Positive Control[a]		30.8 ± 0.8*	23.9 ± 3.4*

a) Ethyl methane sulfonate at 2.5 μM in the without S9 group, cyclophosphamide at 10 μM in the with S9 group; *, $p < 0.05$ versus control.

CAA Induced Micronucleus Formation

Micronuclei formations due to 2-CE and CAA were evaluated using mouse peripheral blood at 12.5, 25, and 50% i.p. of the LD_{50} dose, as previously reported,[24] and no mortality was observed at these dosages. High dose 2-CE (40 mg/kg) induced micronuclei formation, but CAA induced significant increases in micronuclei formation at 0.75, 1.5, and 3 mg/kg dosages (Table 2). Allavena *et al*. (1992) indicated that 2-CE did not exert genotoxic effects when tested *in vivo* with micronucle-

Table 2. Micronucleus Induction in ICR Mice Peripheral Blood Treated with 2-CE and CAA

Treatment		Time (h)	MN RET (%)	MN NEC (%)
Negative Control		48	0.28 ± 0.30	0.28 ± 0.20
		72	0.86 ± 0.26	0.38 ± 0.20
2-CE (mg/kg)	10	48	0.66 ± 0.27	0.28 ± 0.24
		72	1.08 ± 0.19	0.30 ± 0.00
	20	48	0.90 ± 0.21	0.20 ± 0.07
		72	1.40 ± 0.78	0.42 ± 0.16
	40	48	1.28 ± 1.54	0.14 ± 0.15
		72	1.84 ± 0.38*	0.28 ± 0.08
CAA (mg/kg)	0.75	48	0.64 ± 0.17	0.26 ± 0.05
		72	2.64 ± 0.45*	0.30 ± 0.10
	1.5	48	1.36 ± 0.09*	0.50 ± 0.37
		72	3.70 ± 0.92*	0.42 ± 0.27
	3	48	1.66 ± 0.34*	0.26 ± 0.19
		72	5.16 ± 0.78*	1.20 ± 0.64*
Positive Control[a)]		48	1.62 ± 0.72*	0.28 ± 0.08
		72	0.78 ± 0.19	0.26 ± 0.05

a) Cyclophosphamide 50 mg/kg i.p. *, $p < 0.05$ versus control.

ated polychromatic erythrocytes in the bone marrow or micronucleated hepatocytes.[19] Our results showed that treatment with 2-CE, except at the highest concentration for 72 hr, did not induce micronucleus formation, but CAA did. The micronucleus induced by CAA was repaired after 1 week (data not shown). These results are the first to demonstrate that CAA induced micronucleus formation. However, the mechanisms responsible for CAA mutagenicity require further study.

In conclusion, 2-CE-intoxicated patients are commonly found in Taiwan. Little is known about the mechanisms involved in 2-CE intoxication. This study provides information about farmers' chronic exposure to 2-CE and the importance of its metabolite CAA in inducing chromosome aberration and micronucleus formation.

REFERENCES

1) Deng, J. F., Yang, C. C., Tsai, W. J., Ger, J. and Wu, M. L. (2001) Acute ethylene chlorohydrine poisoning: experience of a poison control center. *J. Toxicol. Clin. Toxicol.*, **39**, 587–593.
2) Sood, C. and O'Brien, P. J. (1993) Molecular mechanisms of chloroacetaldehyde-induced cytotoxicity in isolated rat hepatocytes. *Biochem. Pharmacol.*, **46**, 1621–1626.
3) Chen, Y. T., Liao, J. W. and Hung, D. Z. (2010) Protective effects of fomepizole on 2-chloro-ethanol toxicity. *Hum. Exp. Toxicol.*, **29**, 507–512.
4) Gothe, R., Calleman, C. J., Ehrenberg, L. and Wachtmeister, C. A. (1974) Trapping with 3,4-dichlorobenethiol of reactive metabolites formed in vitro from the carcinogen, vinyl chloride. *Ambio*, **3**, 234–236.
5) Huberman, E., Bartsch, H. and Sachs, L. (1975) Mutation induction in Chinese hamster V79 cells by two vinyl chloride metabolites, chloroethylene oxide and 2-chloroacetaldehyde. *Int. J. Cancer*, **16**, 639–644.
6) McCann, J., Simmon, V., Streitwieser, D. and Ames, B. N. (1975) Mutagenicity of chloroacetaldehyde, a possible metabolic product of 1,2-dichloroethane (ethylene dichloride), chloroethanol (ethylene chlorohydrin), vinyl chloride, and cyclophosphamide. *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 3190–3193.
7) Malaveille, C., Bartsch, H. and Barbin, A. (1975) Mutagenicity of vinyl chloride, chloro-ethylene oxide, chloroacetaldehyde and chloroethanol. *Biochem. Biophys. Res. Commun.*, **63**, 363–370.
8) Rannung, U., Gothe, R. and Wachtmeister, C. A. (1976) The mutagenicity of chlororthylene oxide, chloroacetaldehyde, 2-chloroethanol and chloroacetic acid, conceivable metabolites of vinly chloride. *Chem. Biol. Interact.*, **12**, 251–263.
9) Crebelli, R., Conti, G., Conti, L. and Carere, A. (1990) Chloroacetaldehyde is a powerful inducer of mitotic aneuploidy in Aspergillus nidulans. *Mutat. Res.*, **155**, 165–168.
10) Barrio, J. R., Secrist, J. A., III and Leonard, N. J. (1972) Fluorescent adenosine and cytidine derivatives. *Biochem. Biophys. Res. Commun.*, **46**, 597–604.
11) Leonard, N. J. (1984) Etheno-substituted nucleotides and coenzymes: fluorescence and biological activity. *CRC Crit. Rev. Biochem.*, **15**, 125–199.
12) Fucic, A., Garaj-Vrhovac, V., Dimitrovic, B. and Skara, M. (1992) The persistence of sister-chromatid exchange frequencies in men occupationally exposed to vinyl chloride monomer. *Mutat. Res.*, **281**, 129–132.
13) Kandala, J. C., Mrema, J. E. K., DeAngelo, A., Daniel, F. B. and Guntaka, R. V. (1990) 2-Chloroacetaldehyde and 2-chloroethanol are potent inhibitors of DNA synthesis in animal cells. *Biochem. Biophys. Res. Commun.*, **167**, 457–463.
14) Spengler, S. J. and Singer, B. (1988) Formation of interstrand cross-links in chloro-acetaldehyde-treated DNA demonstrated by ethidium bromide fluorescence. *Cancer Res.*, **48**, 4804–4806.

15) Singhal, R. and Landes, J. P. (1988) High performance liquid chromatographic analysis of DNA composition and DNA modification by chloroacetaldehyde. *J. Chromatogr.*, **458**, 117–128.
16) Barbin, A., Bresil, H., Croisy, A., Jacquignon, P., Malaveille, C., Montesano, R. and Bartsch, H. (1975) Liver microsome mediated formation of alkylating agents from vinyl bromide and vinyl chloride. *Biochem. Biophys. Res. Commun.*, **16**, 596–603.
17) Johnson, M. K. (1967) Metabolism of chloroethanol in the rat. *Biochem. Pharmacol.*, **16**, 185–199.
18) OECD (1997) In vitro mammalian chromosome aberration test. Section 4: Health Effect. In: *Guideline for the Testing of Chemicals*, p.473.
19) Allavena, A., Martelli, A., Robbiano, L. and Brambilla, G. (1992) Evaluation in a battery of in vivo assays of four in vitro genotoxins proved to be noncarcinogens in rodents. *Teratog. Carcinog. Mutagen.*, **12**, 31–41.
20) Chanda, S., Erexson, G., Riach, C., Innes, D., Stevenson, F., Murli, H. and Bley, K. (2004) Genotoxicity studies with pure trans-capsaicin. *Mutat. Res.*, **557**, 85–97.
21) Aydemir, N. and Bilaloğlu, R. (2004) The investigation of the genotoxic effects of fenarimol and propamocarb in mouse bone marrow in vivo. *Toxicol. Lett.*, **147**, 73–78.
22) Ivett, J. L., Brown, B. M., Rodgers, C., Anderson, B. E., Resnick, M. A. and Zeiger, E. (1989) Chromosomeal aberrations and sister chromatid exchange tests in Chinese hamster ovart cells in vitro. IV. Results with 15 chemicals. *Environ. Mol. Mutagen.*, **14**, 165–187.
23) Chiang, S. Y., Swenberg, J. A., Weisman, W. H. and Skopek, T. R. (1997) Mutatgenicity of vinyl chloride and its reactive metabolites, chloroethylene oxide and chloroacetaldehyde, in a metabolically competent human B-lymphoblastoid line. *Carcinogenesis*, **18**, 31–36.
24) Lawrence, W. H., Dillingham, E. O., Turner, J. E. and Autian, J. (1972) Toxicity profile of choroacetaldehyde. *J. Pharm. Sci.*, **61**, 19–25.