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The role of oxidative stress in acrolein-induced DNA damage in HepG2 cells

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Abstract

This study evaluated the role of oxidative stress in acrolein-induced DNA damage, using HepG2 cells. Using the standard single cell gel electrophoresis (SCGE) assay, a significant dose-dependent increment in DNA migration was detected at lower concentrations of acrolein; but at the higher tested concentrations, a reduction in the migration was observed. Post-incubation with proteinase K significantly increased DNA migration in cells exposed to higher concentrations of acrolein. These results indicated that acrolein caused DNA strand breaks and DNA-protein crosslinks (DPC). To elucidate the oxidatively generated DNA damage mechanism, the 2,7-dichlorofluorescein diacetate (DCFH-DA) and *o*-phthalaldehyde (OPT) were used to monitor the levels of reactive oxygen species (ROS) and glutathione (GSH), respectively. The present study showed that acrolein induced the increased levels of ROS and depletion of GSH in HepG2 cells. Moreover, acrolein significantly caused 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) formation in HepG2 cells. These results demonstrate that the DNA damage induced by acrolein in HepG2 cells is related to the oxidative stress.

Keywords: *Acrolein, single cell gel electrophoresis, DNA-protein crosslinks, oxidative stress, HepG2 cells*

Abbreviations: *DPC, DNA-protein crosslinks; SCGE, single cell gel electrophoresis; DCFH-DA, 2,7-dichlorofluorescein diacetate; OPT,* o*-phthalaldehyde; ROS, reactive oxygen species; GSH, glutathione; 8-oxodGuo, 8-oxo-7,8-dihydro-2′-deoxyguanosine; IARC, International Agency for Research on Cancer; ALS, alkali labile sites; DDC, DNA-DNA crosslinks.*

Introduction

Acrolein is found widely in the environment, particularly as a component of smoke [1,2]. In the biological system, it is a metabolic product of allyl alcohol, allylamine, spermine, spermidine and chemotherapeutic agent cyclophosphamide [1,3–5]. Acrolein has also been identified as both a product and initiator of lipid peroxidation [6].

Acrolein is an intense irritant and displays a range of toxic effects. The International Agency for Research on Cancer (IARC) concluded that there was inadequate evidence for its carcinogenicity in experimental animals [7]. In addition, there is increasing evidence that acrolein is genotoxic. In the Ames assay for mutagenicity, both positive and negative results were reported [8,9]. Furthermore, DNA strand breaks were detected in skin fibroblasts [10], bronchial epithelial cells [11] and leukemia cells [12]. DNA-protein crosslinks (DPC) were also formed when bronchial epithelial cells were exposed to acrolein [11]. Sister chromatid exchanges and structural chromosomal aberrations were observed in CHO cells [13]. The genotoxic effects of acrolein have already been demonstrated in many cell lines.

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However, this is the first evidence of acrolein-induced DNA damage in human HepG2 cells. The human hepatoma line (HepG2) retains the activities of several phase I and II xenobiotic metabolizing enzymes presented in human hepatocytes [14]. It has been shown to be a suitable system for investigation of genotoxicity [15].

Acrolein adducts have been proposed as a biologic marker for oxidative stress [16]. Accordingly, acrolein levels have been found increased in pathological conditions associated with oxidative stress, such as diabetic nephropathy [17] and Alzheimer's disease [18]. It is currently believed that acrolein mediates many detrimental effects associated with oxidative stress [1,16,19]. Previous studies demonstrated that acrolein was able to induce the generation of reactive oxygen species (ROS) in some cell types, such as PC12 cells [20], bronchial epithelial cells [21] and umbilical vein endothelial cells [22]. In addition, both *in vitro* and *in vivo* studies showed that acrolein caused a significant reduction of intracellular GSH [21–24].

The overall object of the present study is to further explore the role of oxidative stress in acrolein-induced DNA damage, using HepG2 cells. The DNA damage induced by acrolein was measured by the standard and proteinase K-modified alkaline single cell gel electrophoresis (SCGE) assay, which is a very sensitive method for detecting DNA strand breaks, alkali labile sites (ALS), DNA–DNA crosslinks (DDC) and DPC [25]. Since the molecular mechanisms may involve the generation of ROS and depletion of GSH, we monitored the levels of intracellular ROS and GSH using the 2,7-dichlorofluorescein diacetate (DCFH-DA) and *o*-phthalaldehyde (OPT), respectively. 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo), a major form of oxidatively generated DNA damage product which can be generated by ROS [26], was evaluated by immunoperoxidase staining analysis.

Materials and methods

Chemicals, materials and mediums

Acrolein (CAS No.107–02–8) was purchased from Kelong Chemical Reagent Factory (ChengDu, China, purity > 95%). RNase A, Cytochalasin B, DCFH-DA and OPT were obtained from Sigma (St. Louis, MO). Monoclonal 8-oxodGuo antibody and the Ultrasensitive Streptavidin-Peroxidase Kit were from JaICA (Fukuroi, Japan) and Maixin-Bio (FuJian, China). Normal melting point (NMP) agarose and low melting point (LMP) agarose were provided by Gibco BRL, Life Technologies (Paisley, UK). Proteinase K was obtained from Takara-Bio (Dalian, China). All tissue culture reagents, i.e. minimal essential Eagle's medium, foetal bovine serum, antibiotics (penicillin, streptomycin) and trypsin-EDTA were bought from Gibco BRL-Life Technologies (Grand Island, NY).

Cell culture and treatment

The human hepatoma line (HepG2) was supplied by the American Type Culture Collection (ATCC, HB-8065). The HepG2 cells were grown in minimum essential Eagle's medium containing 10% (v/v) foetal bovine serum and antibiotics (penicillin (100 IU/ml) and streptomycin (100 μg/ml)) at 37°C in 5% CO_2. The cells were used at passages between 7 and 9. The stock solution (50 mM) of acrolein was made fresh in water on the day of use on ice. The cells were treated with different concentrations of acrolein (final concentration: 12.5, 25, 50 and 100 μM). Control received only minimum essential Eagle's medium. Acrolein was dissolved in the culture medium just before use.

SCGE assay

The SCGE assay was performed as described by Singh and Stephens [27], with slight modifications. Cells were exposed to acrolein and hydrogen peroxide (H_2O_2) (for positive control, 20 μM) for 1 h at 37°C. To avoid artifacts resulting from apoptosis and necrosis, Hoechst 33342 (8 μg/ml) and trypan blue (50 μg/ml) were employed to detect the apoptotic cells and cell viability. Only cell suspensions with viabilities > 90% and no apoptotic cell were used to determine DNA migration on gels. Cell suspension was mixed with 1% LMP agarose and added to fully frosted slides that had been covered with a layer of 1.5% NMP agarose. The cells were then lysed for 1 h at 4°C in a buffer consisting of 2.5 M NaCl, 100 mM EDTA, 1% Triton X-100 and 10 mM Tris, pH 10. After lysis, the slides were placed on an electrophoresis unit filled with fresh electrophoretic buffer (300 mM NaOH and 1 mM EDTA, pH 13) and left for 20 min for DNA unwinding and then electrophoresed for 30 min at 18 V and 200 mA. Afterwards, the slides were neutralized with 0.4 M Tris buffer (pH 7.5) and stained with 50 μl of ethidium bromide (20 μg/ml). Finally, the slides were viewed using an Olympus BX-51 fluorescent microscope (excitation filter 549 nm, barrier filter 590 nm). Images of 50 randomly selected cells from each slide were analysed with Comet Assay Software Project casp-1.2.2 (University of Wroclaw, Poland). Three independent experiments were carried out in every case. The following parameters were evaluated: tail length (μm), tail DNA (%) and tail moment (TM).

Detection of DPC

Slides were incubated with proteinase K according to the method of Merk et al. [28]. The slides after lysis were washed three times (5 min, 4°C) in TE buffer

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containing 10 mM Tris, 1 mM EDTA, pH 10 and drained. Aliquots of 50 μl of TE buffer or 50 μl of 1 mg/ml proteinase K in TE buffer were applied to slides. Slides were incubated for 2 h at 37°C in a moist chamber. Further steps were as described above for the SCGE assay. The presence of DPC is indicated by an increase in DNA migration in the acrolein-treated cells after proteinase K post-treatment compared to cells treated without proteinase K.

Intracellular ROS formation

The formation of intracellular ROS was measured using a fluorescent probe DCFH-DA as described previously [29]. The principle of the test is based upon the fluorescent probe DCFH-DA diffusing into the cells through cell membrane. Then, the DCFH-DA is hydrolysed to non-fluorescent 2′,7′-dichlorofluorescin (DCFH). ROS cause oxidation of DCFH to a measurable fluorescent product, DCF. The DCF fluorescence intensity is proportional to the amount of ROS formed intracellularly. H_2O_2 is the principle ROS responsible for the oxidation of DCFH-DA to DCF [30].

Briefly, the cells were harvested and treated with acrolein and H_2O_2 (for positive control, 20 μM) at 37°C for 1 h. Following treatment, cells were washed with cold PBS, suspended in PBS at 5×10^5 cells/ml and incubated with DCFH-DA at a final concentration of 5 μM (40 min, 37°C in darkness). The relative fluorescence intensity was monitored using a fluorescence spectrophotometer (HITACHI, 650–60, Tokyo, Japan, excitation wavelength of 485 nm, emission wavelength of 530 nm).

Measurement of intracellular GSH

Reduced glutathione (GSH) was measured using a modified method of Hissin and Hilf [31]. The cells were exposed to acrolein at 37°C for 1 h, washed twice with PBS and then 5% trichloroacetic acid (TCA) (0.4 ml) was added. After incubation at 4°C for 30 min to extract GSH, 50 μl of the TCA extract was mixed with 0.8 ml 50 mM phosphate/5 μM EDTA buffer (pH 8) and the reaction was initiated by the addition of 50 μl OPT (1 mg/ml). The mixture was incubated at 37°C for 15 min in darkness. Fluorescence intensity was monitored by a fluorescence spectrophotometer (HITACHI 650-60, Tokyo, Japan, excitation wavelength of 350 nm, emission wavelength of 420 nm). Fluorescence intensity was corrected with the appropriate control sample containing only OPT.

Immunoperoxidase staining for 8-oxodGuo

The cells were treated with acrolein at 37°C for 3 h on a coverslip and rinsed with PBS twice. Level of 8-oxodGuo was determined by the immunocytochemical technique as Yarborough et al. [32] described with minor modifications. The images were recorded by microscope (Olympus BX-51, Omachi, Japan). The relative intensity of nuclear staining of 50 randomly selected cells was quantified by a multi-parameter image analysis program, Image-Pro Plus 4.5.1. The staining data represented the average absorbance multiplied by 1000.

MTT assay for cell viability

HepG2 cells were plated in a 96-well microtiter plate at a density of 1×10^4 cells per well in a final volume of 100 μl minimum essential Eagle's medium. The cells were treated with different concentrations of acrolein for 24 h. The effect of acrolein on cytotoxicity in HepG2 cells was determined by the methyl thiazol tetrazolium bromide (MTT) assay [33]. Cell viability was calculated by comparing the opitical density of culture given a particular treatment with that of the untreated control.

Statistical analysis

Statistical analysis was performed using SPSS v13.0 software. Data are expressed as mean ± standard deviation (SD). The statistical significance of differences among groups was determined by analysis of variance with one-way ANOVA, followed by least significant difference (LSD) for multiple comparison, as a post hoc test. For the result of modified SCGE assay, difference between two treatment groups was compared by the Student's *t*-test. The level of significance was set at $p < 0.05$ and $p < 0.01$ for all statistical analysis.

Results

Induction of DNA damage

Table I shows the numerical values for tail length, tail DNA (%) and TM in HepG2 cells exposed to different concentrations of acrolein and the positive control (H_2O_2, 20 μM) for 1 h. In all groups, the cell viabilities were consistently > 90% and no apoptosis was observed (data not shown). H_2O_2 caused a significant increase of the DNA migration ($p <$ 0.01). The treatment with acrolein at all tested

Table I. Effect of acrolein on DNA migration in HepG2 cells.

Acrolein (μM)	Tail length (μm)	Tail DNA (%)	Tail moment (μm)
0	53.82 ± 23.98	32.09 ± 12.62	19.46 ± 13.72
12.5	126.36 ± 27.83**	61.12 ± 9.15**	78.14 ± 23.63**
25	186.96 ± 21.68**	74.61 ± 6.64**	140.13 ± 24.85**
50	139.88 ± 30.83**	61.50 ± 8.86**	86.94 ± 27.96**
100	94.60 ± 18.39**	50.63 ± 7.58**	48.88 ± 15.48**
H_2O_2	178.54 ± 22.74**	67.09 ± 8.72**	107.08 ± 13.76**

**p < 0.01, significantly different from control.

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concentrations (12.5–100 μM) produced a significant increment in all the considered parameters in relation to control ($p < 0.01$). At lower concentrations of acrolein (12.5 and 25 μM), we observed a significant dose-dependent increment in the DNA migration, whereas at 50 and 100 μM a lower increment was seen compared to the maximum migration at 25 μM. These results showed the induction of DNA strand breaks at lower concentrations (12.5–25 μM) and DNA crosslinks formation at higher concentrations (50–100 μM).

Detection of DPC by proteinase K

To find out whether DPC are actually induced by acrolein, we treated slides with proteinase K after lysis. Table II displays the results for DNA migration in HepG2 cells incubated with acrolein after proteinase K post-treatment compared to cells treated without proteinase K. HepG2 cells incubated with acrolein at 50 and 100 μM and post-treated with proteinase K showed a significant increase ($p < 0.01$) in all considered parameters compared to cells without treatment with this enzyme, suggesting the presence of DPC. Post-treatment with proteinase K had no influence on DNA migration in cells exposed to acrolein at 12.5 and 25 μM.

Induction of ROS by acrolein

To explore the mechanisms of acrolein-DNA damage, we first tested the effect of acrolein on the formation of intracellular ROS in HepG2 cells. With H_2O_2 (20 μM) as the positive control, the result showed a significant difference (23.31 ± 2.21 vs 7.77 ± 3.58, $p < 0.01$) in DCF fluorescence intensity compared to the control. Figure 1 shows that a significant increase of DCF fluorescence intensity was observed in cells treated with acrolein at 50 and 100 μM ($p < 0.05$ or < 0.01). The DCF fluorescence intensity in HepG2 cells at the highest dose of acrolein (100 μM) was ~3-fold higher compared to control.

Depletion of intracellular GSH by acrolein

The effect of different concentrations of acrolein on cellular GSH content in HepG2 cells is shown in Figure 2. After cells were incubated for 1 h with different concentrations of acrolein (25–100 μM), a striking decrease of intracellular GSH was observed in HepG2 cells ($p < 0.01$).

Effect of acrolein on 8-oxodGuo formation

The immunoperoxidase method was used to detect oxidatively generated DNA damage in HepG2 cells treated with acrolein. Figure 3 shows the result of immunoperoxidase staining for 8-oxodGuo in HepG2 cells exposed to different concentrations of acrolein. It can be seen that the staining intensity of 8-oxodGuo at higher concentrations of acrolein (25–100 μM) was different from that observed in control ($p < 0.01$), whereas no damage occurred at lower concentration.

Effect of acrolein-induced cytotoxicity

HepG2 cells were exposed to acrolein (12.5–400 μM) and cell viability was measured by MTT assay. Figure 4 shows the relationship between different concentrations of acrolein and percentage of cells survived. Exposure of HepG2 cells to different concentrations of acrolein resulted in a concentration-dependent decrease in cell viability.

Discussion

In the present study acrolein-induced DNA damage was measured in HepG2 cells and its ability to induce cellular oxidative stress was also evaluated.

The present study showed that acrolein could induce DNA strand breaks and DPC assessed by standard and proteinase K-modified SCGE assays in HepG2 cells. When the standard SCGE assay was performed, a significant dose-dependent increment in all the considered parameters was observed at lower concentrations of acrolein (12.5 and 25 μM).

Table II. Parameters of cells incubated with acrolein and post-treated with proteinase K or only with TE buffer.

Acrolein (μM)	PI	Tail length (μm)	Tail DNA (%)	Tail moment (μm)
0	TE	31.18 ± 9.70	18.91 ± 7.34	6.58 ± 4.53
	PK	32.52 ± 8.54	20.73 ± 6.69	6.94 ± 3.67
12.5	TE	106.46 ± 24.47	40.85 ± 7.18	44.35 ± 16.20
	PK	109.82 ± 26.87	41.73 ± 6.45	46.82 ± 16.85
25	TE	112.48 ± 20.67	46.83 ± 8.67	53.91 ± 18.40
	PK	118.50 ± 24.78	45.32 ± 6.32	53.48 ± 18.71
50	TE	91.02 ± 19.63	40.09 ± 8.12	37.87 ± 14.79
	PK	175.08 ± 35.48**	55.09 ± 12.97**	100.28 ± 39.18**
100	TE	41.24 ± 7.26	30.18 ± 9.16	12.93 ± 5.29
	PK	186.16 ± 22.36**	71.88 ± 11.27**	134.82 ± 31.67**

PI = post-incubation with TE buffer or proteinase K.
**$p < 0.01$, significant differences between proteinase K treatment and the non-treated groups (TE).

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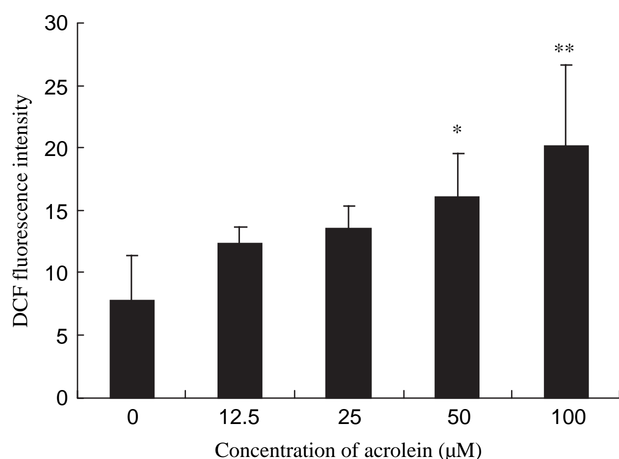

Figure 1. Effect of different concentrations of acrolein (12.5–100 μM) on ROS production in HepG2 cells monitored by using the DCFH-DA assay. Each bar represents the mean ± SD obtained in three independent experiments ($n = 3$); * and ** indicate statistical significance as compared to control, $p < 0.05$ and $p < 0.01$.

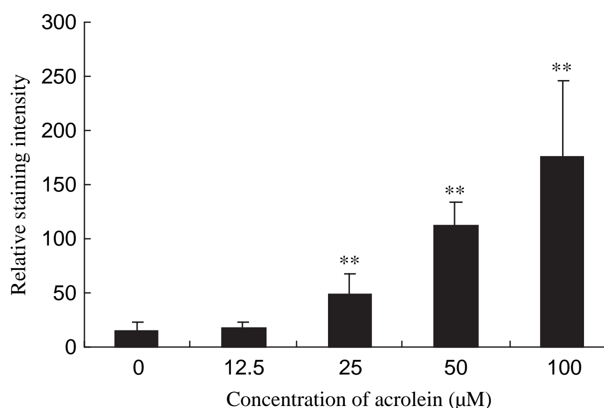

Figure 3. Effect of acrolein on oxidatively generated DNA damage evaluated by the determination of the staining intensity of 8-oxodGuo. HepG2 cells were exposed to increasing concentrations of the compound (12.5–100 μM) for 3 h. Each bar represents the mean ± SD of three independent experiments ($n = 3$); ** indicates statistical significance as compared to control, $p < 0.01$.

However, cells treated with higher concentrations of acrolein (50–100 μM) presented a lower increment than the maximum DNA migration at 25 μM. These results showed the induction of DNA strand breaks at lower concentrations and DNA crosslinks formation at higher concentrations, therefore a modification of the standard SCGE assay was performed. Incubation with proteinase K allowed for the release of proteins joined to the DNA and consequently an increase in DNA migration at the higher concentrations of acrolein (50–100 μM) was observed. In contrast post-treatment with proteinase K had no influence on DNA migration in cells exposed to lower concentrations of acrolein (12.5 and 25 μM). Therefore, our study indicates that acrolein induces a clear dose-dependent shift from DNA strand breaks to DPC.

Our results appear to be inconsistent with the results presented in the literatures in relation to the standard SCGE assay by acrolein. It was mentioned that acrolein at lower concentrations (≤ 50 μM) did not induce DNA damage, whereas acrolein at 100 and 500 μM induced an increase of the DNA migration in Raji cells. These results indicate DNA strand breaks in cells exposed to acrolein at higher concentrations [34]. Another study demonstrated that treatment of rat hepatocytes with acrolein (0.5–2 mg/ml) resulted in TM which was not different from those of control values. The study also showed that acrolein caused characteristic DNA spots image with small, highly condensed areas within the otherwise circular DNA spots. The condensed areas are probably the consequence of known DNA and protein cross-linking activities of acrolein [35]. In our study, we indicate the induction of DNA strand

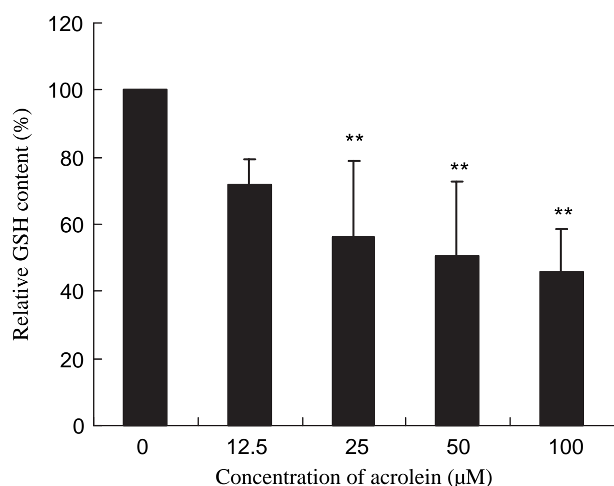

Figure 2. Effect of different concentrations of acrolein (12.5–100 μM) on the GSH content in HepG2 cells exposed to the compound for 1 h. Each bar represents the mean ± SD of the relative GSH content in three independent experiments ($n = 3$); ** indicates statistical significance as compared to control, $p < 0.01$.

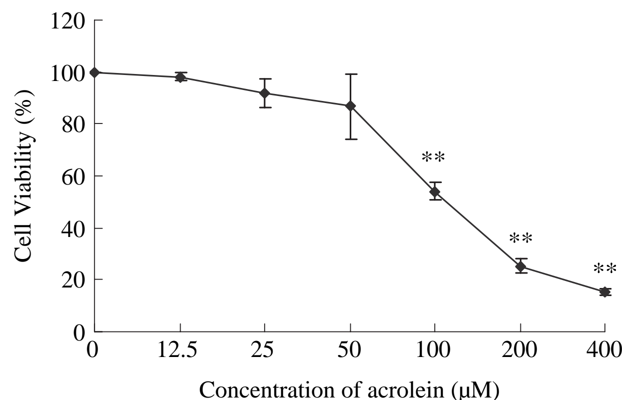

Figure 4. Effect of acrolein-induced cytotoxicity in HepG2 cells measured by MTT assay. The cells were treated with different concentrations of acrolein for 24 h. Values are the mean ± SD of three independent experiments ($n = 3$); ** indicates statistical significance as compared to control, $p < 0.01$.

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breaks at lower concentrations and DPC formation at higher concentrations.

The SCGE assay is a sensitive test for the measurement of DNA damage in individual cells. In contrast to many different DNA damage that lead to increased DNA migration in the SCGE assay, DPC and DDC are the only known DNA modifications that cause an actual decrease in DNA migration. In the present study, proteinase K-modified SCGE assay showed the ability of acrolein to induce the formation of DPC in HepG2 cells. However, we cannot exclude a contribution of inter- and intra-strand DDC observed by acrolein [36].

Using DCFH as a fluorescene probe, our result clearly showed that the formation of intracellular ROS was significantly increased in cells exposed to acrolein at higher concentrations (50–100 μM). ROS can damage DNA and lead to genotoxicity [37], it is possible to infer that ROS may play an important role in the induction of acrolein-DNA damage. In addition, acrolein may exert its detrimental effect through ROS formation and lipid peroxidation [16,21,38]. Mitochondria are one of the most important cellular sources of ROS production and are particularly susceptible to oxidative stress [39]. It has been shown that acrolein-induced mitochondrial oxidative stress is due to the increased production of ROS [20,40].

GSH is the major intracellular antioxidant, which serves as a substrate for GSH peroxidase to degrade hydrogen peroxide to H_2O and also acts as a free radical scavenger [41]. In the present study, a decrease of intracellular GSH was observed in HepG2 cells exposed to higher concentrations of acrolein (25–100 μM). Acrolein can react with the sulphydryl group of GSH via a Michael-type addition reaction, resulting in the formation of covalently bound aldehyde group and the loss of sulphydryl [1]. The studies of Eisenbrand et al. [42] suggest that intracellular GSH may protect against the DNA damage induced by acrolein. These observations indicate that acrolein can enhance oxidative stress by disrupting intracellular GSH.

8-oxodGuo is generally used as an index to examine the oxidatively generated DNA damage [43]. It can pair with adenine as well as cytosine and cause G:C to T:A transversions during DNA replication [44]. Our result demonstrated that acrolein at the highest tested concentration (25–100 μM) was capable of inducing a dose-dependent increase in the staining intensity of 8-oxodGuo in HepG2 cells. Our data do not exclude inhibition in the repair of this damage. For example, Yang et al. [34] reported previously that acrolein inhibits the repair of gamma-irradiation-induced DNA damage.

In our study, we suggest that oxidative stress is, at least in part, responsible for DNA damage caused by acrolein. However, another evidence indicates that acrolein-DNA adducts exist as intermediates along a pathway of formation for DPC [45]. In view of the reactivity of acrolein, the most likely mechanism for the formation of DPC is alkylation. Further study is needed to determine whether oxidative stress induced by acrolein is related to the formation of DPC.

Oxidative stress refers to a serious imbalance between production of reactive species and antioxidant defence [43]. There are several hypotheses which can be put forward for the origin of the observed oxidative stress. For instance, acrolein induced the generation of oxygen radicals, such as superoxide anion and hydroxyl radicals in the presence of xanthine oxidase [16,46]. Acrolein induced diminished levels of antioxidants, such as GSH. In addition, oxidizing properties of acrolein could be responsible for the oxidative stress, although the underlying mechanism is not clear.

Acrolein has been shown to interact with nucleophiles, including DNA and protein in cells [1]. The major adduct generated by the reaction of acrolein with DNA is 8-hydroxy-1,N^2-propano-2′-deoxyguanosine [47]. Immunoassays have demonstrated 1,N^2-propano-2′-deoxyguanosine adducts in the DNA of *Salmonella typhimurium* tester strains and cultured CHO cells exposed to acrolein [48,49]. These adducts were also detected in DNA extracted from rat and human liver [47,50].

The mechanisms of mutagenicity of acrolein have been examined previously in *Salmonella typhimurium* [48] and *Escherichia coli* [51], where it has been shown to induce the formation of DNA adducts in a dose-dependent manner. Acrolein-DNA adducts induce mainly G:C to T:A transversions in human DNA, the major type of mutations found in the p53 gene in cigarette smoke-related lung cancer [52]. Together with the results of our present study, we infer that various mechanisms may contribute to acrolein-induced DNA damage.

In conclusion, we conclude that the DNA damage of acrolein is mediated by the formation of ROS and depletion of GSH, which cause oxidatively generated DNA damage, formation of DNA strand breaks and DPC.

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***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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References

[1] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 1991;11:81–128.

[2] O'Brien PJ, Siraki AG, Shangari N. Aldehyde sources, metabolism, molecular toxicity mechanisms, and possible effects on human health. Crit Rev Toxicol 2005;35:609–662.

[3] Nelson TJ, Boor PJ. Allylamine cardiotoxicity-IV. Metabolism to acrolein by cardiovascular tissues. Biochem Pharmacol 1982;31:509–514.

[4] Sakata K, Kashiwagi K, Sharmin S, Ueda S, Igarashi, K. Acrolein produced from polyamines as one of the uraemic toxins. Biochem Soc Trans 2003;31:371–374.

[5] Gurtoo HL, Marinello AJ, Struck RF, Paul B, Dahms RP. Studies on the mechanism of denaturation of cytochrome P-450 by cyclophosphamide and its metabolites. J Biol Chem 1981;256:11691–11701.

[6] Uchida K, Kanematsu M, Morimitsu Y, Osawa T, Noguchi N, Niki E. Acrolein is a product of lipid peroxidation reaction. Formation of free acrolein and its conjugate with lysine residues in oxidized low density lipoproteins. J Biol Chem 1998;273:16058–16066.

[7] IARC Acrolein. IARC Monogr 1995;63:337–372.

[8] Hales BF. Comparison of the mutagenicity and teratogenicity of cyclophosphamide and its active metabolites,4-hydroxycyclophosphamide, phosphoramide mustard, and acrolein. Cancer Res 1982;42:3016–3021.

[9] Marnett LJ, Hurd HK, Hollstein MC, Levin DE, Esterbauer H, Ames BN. Naturally occurring carbonyl compounds are mutagens in Salmonella tester strain TA104. Mutat Res 1985;148:25–34.

[10] Dypbukt JM, Atzori L, Edman CC, Grafstrom RC. Thiol status and cytopathological effects of acrolein in normal and xeroderma pigmentosum skin fibroblasts. Carcinogenesis 1993;14:975–980.

[11] Grafstrom RC, Dypbukt JM, Willey JC, Sundqvist K, Edman C, Atzori L, Harris CC. Pathobiological effects of acrolein in cultured human bronchial epithelial cells. Cancer Res 1988;48:1717–1721.

[12] Crook TR, Souhami RL, Mclean AE. Cytotoxicity, DNA cross-linking and single strand breaks induced by activated cyclophosphamide and acrolein in human leukemia cells. Cancer Res 1986;46:5029–5034.

[13] Au W, Sokova OI, Kopnin B, Arrighi FE. Cytogenetic toxicity of cyclophosphamide and its metabolites *in vitro*. Cytogenet Cell Genet 1980;26:108–116.

[14] Knasmuller S, Parzefall W, Sanyal R, Ecker S, Schwab C, Uhl M, Mersch-Sundermann V, Williamson G., Hietsch G, Langer T, Darroudi F, Natarajan AT. Use of metabolically competent human hepatoma cells for the detection of mutagens and antimutagens. Mutat Res 1998;402:185–202.

[15] Knasmuller S, Mersch-Sundermann V, Kevekordes S, Darroudi F, Huber WW, Hoelzl C, Bichler J, Majer BJ. Use of a human-derived liver cell lines for the detection of environmental and dietary genotoxicants; current state of knowledge. Toxicology 2004;198:315–328.

[16] Kehrer JP, Biswal SS. The molecular effects of acrolein. Toxicol Sci 2000;57:6–15.

[17] Suzuki D, Miyata T. Carbonyl stress in the pathogenesis of diabetic nephropathy. Intern Med 1999;38:309–314.

[18] Calingasan NY, Uchida K, Gibson GE. Protein-bound acrolein: a novel marker of oxidative stress in Alzheimer's disease. J Neurochem 1999;72:751–756.

[19] Uchida K. 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. Prog Lipid Res 2003;42:318–343.

[20] Luo J, Robinson JP, Shi R. Acrolein-induced cell death in PC12 cells: role of mitochondria-mediated oxidative stress. Neurochem Int 2005;47:449–457.

[21] Nardini M, Finkelstein EI, Reddy S, Valacchi G, Traber M, Cross CE, van der Vliet A. Acrolein-induced cytotoxicity in cultured human bronchial epithelial cells. Modulation by alpha-tocopherol and ascorbic acid. Toxicology 2002;170: 173–185.

[22] Park YS, Misonou Y, Fujiwara N, Takahashi M, Miyamoto Y, Koh YH, Suzuki K, Taniguchi N. Induction of thioredoxin reductase as an adaptive response to acrolein in human umbilical vein endothelial cells. Biochem Biophys Res Commun 2005;327:1058–1065.

[23] Zitting A, Heinonen T. Decrease of reduced glutathione in isolated rat hepatocytes caused by acrolein, acrylonitrile, and the thermal degradation products of styrene copolymers. Toxicology 1980;17:333–341.

[24] Arumugam N, Thanislass J, Ragunath K, Niranjali S, Devaraj H. Acrolein-induced toxicity-defective mitochondrial function as a possible mechanism. Arch Environ Contam Toxicol 1999;36:373–376.

[25] Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF. Single cell gel/comet assay: guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environ Mol Mutagen 2000;35: 206–221.

[26] Kasai H. Analysis of a form of oxidative DNA damage, analysis of a form of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. Mutat Res 1997;387:147–163.

[27] Singh NP, Stephens RE. Microgel electrophoresis: sensitivity, mechanisms, and DNA electrostretching. Mutat Res 1997; 383:167–175.

[28] Merk O, Reiser K, Speit G. Analysis of chromate-induced DNA-protein crosslinks with the comet assay. Mutat Res 2000;471:71–80.

[29] Sohn JH, Han KL, Lee SH, Hwang JK. Protective effects of panduratin A against oxidative damage of tert-butylhydroperoxide in human HepG2 cells. Biol Pharm Bull 2005;28:1083–1086.

[30] LeBel CP, Ischiropoulos H, Bondy SC. Evaluation of the probe 2′,7′-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. Chem Res Toxicol 1992;5:227–231.

[31] Hissin PJ, Hilf R. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal Biochem 1976;74:214–226.

[32] Yarborough A, Zhang YJ, Hsu TM, Santella RM. Immunoperoxidase detection of 8-hydroxydeoxyguanosine in aflatoxin B1-treated rat liver and human oral mucosal cells. Cancer Res 1996;56:683–688.

[33] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55–63.

[34] Yang Q, Hergenhahn M, Weninger A, Bartsch H. Cigarette smoke induces direct DNA damage in the human B-lymphoid cell line Raji. Carcinogenesis 1999;20:1769–1775.

[35] Kuchenmeister F, Schmezer P, Engelhardt G. Genotoxic bifunctional aldehydes produce specific images in the comet assay. Mutat Res 1998;419:69–78.

[36] Sanchez AM, Kozekov ID, Harris TM. Lloyd RS. Formation of inter- and intrastrand imine type DNA-DNA cross-links through secondary reactions of aldehydic adducts. Chem Res Toxicol 2005;18:1683–1690.

[37] Epe B. Genotoxicity of singlet oxygen. Chem Biol Interact 1991;80:239–260.

[38] Uchida K. Current status of acrolein as a lipid peroxidation product. Trends Cardiovasc Med 1999;9:109–113.

[39] Cadenas E, Davies KJ. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med 2000;29: 222–230.

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[40] Luo J, Shi R. Acrolein induces oxidative stress in brain mitochondria. Neurochem Int 2005;46:243–252.

[41] Nappi AJ, Vass E. Comparative studies of enhanced iron-mediated production of hydroxyl radical by glutathione, cysteine, ascorbic acid, and selected catechols. Biochim Biophys Acta 1997;1336:295–302.

[42] Eisenbrand G, Schuhmacher J, Golzer P. The influence of glutathione and detoxifying enzymes on DNA damage induced by 2-alkenals in primary rat hepatocytes and human lymphoblastoid cells. Chem Res Toxicol 1995;8:40–46.

[43] Halliwell B, Whiteman M. Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean. Br J Pharmacol 2004; 142:231–255.

[44] Moriya M. Single-stranded shuttle phagemid for mutagenesis studies in mammalian cells: 8-oxoguanine in DNA induces targeted G.C→T.A transversions in simian kidney cells. Proc Natl Acad Sci USA 1993;90:1122–1126.

[45] Kurtz AJ, Lloyd RS. 1,N^2-deoxyguanosine adducts of acrolein, crotonaldehyde, and trans-4-hydroxynonenal cross-link to peptides via Schiff base linkage. J Biol Chem 2003; 278:5970–5976.

[46] Adams JD, Klaidman LK. Acrolein-induced oxygen radical formation. Free Radic Biol Med 1993;15:187–193.

[47] Nath RG, Ocando JE, Chung FL. Detection of 1, N^2-propanodeoxyguanosine adducts as potential endogenous DNA lesions in rodent and human tissues. Cancer Res 1996; 56:452–456.

[48] Foiles PG, Akerkar SA, Chung FL. Application of an immunoassay for cyclic acrolein deoxyguanosine adducts to assess their formation in DNA of Salmonella typhimurium under conditions of mutation induction by acrolein. Carcinogenesis 1989;10:87–90.

[49] Foiles PG, Akerkar SA, Miglietta LM, Chung FL. Formation of cyclic deoxyguanosine adducts in Chinese hamster ovary cells by acrolein and crotonaldehyde. Carcinogenesis 1990; 11:2059–2061.

[50] Nath RG, Chung FL. Detection of exocyclic 1, N^2-propanodeoxyguanosine adducts as common DNA lesions in rodents and humans. Proc Natl Acad Sci USA 1994;91:7491–7495.

[51] Parent RA, Caravello HE, San RH. Mutagenic activity of acrolein in S. typhimurium and E. coli. J Appl Toxicol 1996; 16:103–108.

[52] Feng Z, Hu W, Hu Y, Tang MS. Acrolein is a major cigarette-related lung cancer agent: preferential binding at p53 mutational hotspots and inhibition of DNA repair. Proc Natl Acad Sci USA 2006;103:15404–15409.

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