Review

Genotoxicity of Acrylamide and Glycidamide: A Review of the Studies by *HPRT* Gene and *TK* Gene Mutation Assays

Lin Ao^{1,2} and Jia Cao^{1,2,3}

¹Institute of Toxicology, College of Preventive Medicine, Third Military Medical University, Chongqing, PR China

²Key Laboratory of Electromagnetic Radiation Damage and Medical Protection, Ministry of Education of China, Chongqing, PR China

(Received September 19, 2011; Revised December 6, 2011; Accepted December 28, 2011)

Acrylamide (AA), a proven rodent carcinogen, is found in a variety of commonly consumed human foods, which has raised public health concerns. AA is largely oxidized to the chemically reactive epoxide, glycidamide (GA), by cytochrome P450 2E1. The genotoxic effects of AA and GA have been extensively evaluated. However, the results in mammalian gene mutation tests were inconsistent, especially the genotoxic effects at the *HPRT* gene and *TK* gene. In this article, the relevant mutations induced by AA and GA on both gene loci in various test systems involving *in vivo* and *in vitro* tests are reviewed. It is confirmed that AA acts directly as a clastogen and produces weakly mutagenic effects at the *HPRT* gene probably by metabolic conversion of AA to GA. On the other hand, GA is a strong mutagen with high reactivity to DNA, inducing predominantly point mutations. The molecular mutation spectra of AA and GA at the *HPRT* and *TK* genes are also compared and summarized here, for better clarifying the mechanisms of mutation induced by these two compounds. These data would help to understand the mutagenicity of AA and its contribution to human cancers.

Key words: acrylamide, glycidamide, genotoxicity, *HPRT* mutation, *TK* mutation

Introduction

Acrylamide (AA), a water-soluble vinyl monomer, has been produced since the 1950s by hydration of acrylonitrile (1). It has multiple chemical and industrial applications around the world, such as the use in water treatment, manufacture of paper, plastics and textiles, and polyacrylamide gel preparation in research laboratories (2). It is estimated human exposure from these applications is very limited. More recently, the concerns about the health risks of AA were transferred to the general population from the occupational exposure in the past, since it is found to form naturally during the frying, roasting or baking of carbohydrate-rich foods at

very high temperatures, as a result of Maillard reactions involving asparagine and reducing sugars (3). Total dietary daily AA intake has been estimated to correspond to about 0.5 µg AA per kg body weight on the basis of a normal western diet (4,5). In 2002, a consultation held by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) concluded that AA represented a “major problem”, because of the relative high levels in these foods (6). The finding has prompted renewed interest in its potential for toxicity in humans.

AA exhibits carcinogenicity in experimental animals, causing tumors at multiple organ sites in mice and rats when given in drinking water or by other means (6). However, epidemiologic studies have failed to establish a relationship between AA exposure and an increased risk for cancer, in either occupationally exposed workers or the general populations of several countries in which AA is present in certain foods and beverages (7). In 1994, AA was classified as a probable human carcinogen by the International Agency for Research on Cancer (IARC, Group 2A), based on the significant evidence of carcinogenicity in experimental rodent models and DNA adduct formation in human bronchial cells (8,9). Because of the known human exposure and carcinogenic effects induced by AA in animals, it is critical to understand its potential for inducing genetic damages in animals as well as in humans.

In both humans and experimental animals, AA is largely oxidized to the chemically reactive epoxide, glycidamide (GA), by cytochrome P450 2E1 (CYP2E1) (10). A broad body of evidence has demonstrated the genotoxicity of AA and its reactive metabolite epoxide GA (9,11,12). AA exhibits clastogenic effects by induc-

³Correspondence to: Jia Cao, Institute of Toxicology, College of Preventive Medicine, Third Military Medical University, Chongqing, PR China. Fax: +86-23-68772533, E-mail: caojia1962@126.com

ing structural chromosomal aberrations, micronucleus formations, sister chromatid exchanges and mitotic disturbances in mammalian cells *in vitro* with or without metabolic activation. In the comet assay, AA could induce DNA damage in the FRTL5 and PC Cl3 rat thyroid cell lines (13), as well as in human HepG2 cells (14). In the micronucleus (MN) test, positive results were observed in rodents (15–17). Meanwhile, several studies reported the induction of chromosomal aberrations and mitotic disruptions following AA exposure of cultured mammalian cells, mostly Chinese hamster cell lines (11).

While the direct-acting clastogenic effects of AA have been clearly elucidated, the findings in point mutation assays are inconsistent. As reported by IARC and FAO/WHO, AA did not induce gene mutations in Ames test with or without metabolic activation (18), but GA was always mutagenic in the same assay even in the absence of an exogenous activating system (19). On the other hand, AA has exhibited equivocal, negative, weakly or mildly positive response in mammalian cell gene mutations assays (20). Most of the results above mentioned come from the studies measuring mutations at the specific loci which code thymidine kinase (*TK*) and hypoxanthine-guanine phosphoribosyl transferase (*HPRT*), respectively. In this article, the relevant mutations induced by AA and GA on both gene loci in various test systems are reviewed; these data would help to understand the mutagenicity of AA and its contribution to human cancers.

Mammalian Gene Mutation Assays at the *HPRT* and *TK* Loci

A wide range of assays are now available which enable the effective detection of the mutagenic and more generally genotoxic activity of individual chemicals and mixtures. Among these assays, the mammalian cell gene mutation assays on the *HPRT* gene and the *TK* gene have been widely used to determine the genotoxic potential of various agents. The *HPRT* mutation assay uses the *HPRT* gene as a reporter of mutation. This is a single copy gene at position Xq26–27 in humans, codes for HPRT, an enzyme in the nucleotide salvage pathway (21). Mutations at this locus are recognized phenotypically by resistance to 8-azaguanine (8-AZA) or 6-thioguanine (6-TG), purine nucleotide analogues that selectively kill wild-type cells or cells with normal HPRT activity (22). Similarly, in the *TK* mutation test, cells deficient in thymidine kinase due to the mutation of *TK*^{+/−} to *TK*^{−/−} are resistant to the cytotoxic effects of the pyrimidine analogue trifluorothymidine (TFT). Thus mutant cells are selected in the presence of TFT (23). However, the *HPRT* gene mutation assay and *TK* gene mutation assay detect different spectra of genetic events: the former detects only intragenic mutations such

as point mutations and small deletions (24), while the latter is capable of detecting a wide range of mutational events, including point mutations, large scale chromosomal changes, recombination, aneuploidy and others (25). Additionally, there is a striking feature existing in the *TK* mutant colonies, which is the induction of large colonies growing at normal growth rates and small colonies growing at slower rates. The relative frequency of the two colony classes is mutagen-dependent, with the chemicals that mainly induce gross chromosome aberrations primarily producing small colony mutants and chemicals that mainly induce point mutations primarily forming large colonies (26,27).

A variety of cell types are available for use in *HPRT* and/or *TK* gene mutation assays. In the assessment of mutagenic effects induced by AA and its metabolite GA, the cell subclones employed in *TK* and *HPRT* gene mutation assays include L5178Y *TK*^{+/−} mouse lymphoma cell (18,28–30), human lymphoblastoid cell TK6 (31), Chinese hamster ovary (CHO) cell (32), Chinese hamster V79 cell (33–35) and human promyelocytic leukaemia cells HL-60 and NB4 (36). More recently, the development of the mammalian cell gene assays employing rodent lymphocytes has also contributed significantly to the understanding of *in vivo* somatic cell mutagenesis induced by AA, including the assays performed in Big Blue mice, B6C3F1/*TK* mice and Big Blue rats (37–39).

Mutagenicity of AA and GA at the *HPRT* Locus in Different Test Systems

GA is consistently mutagenic at *HPRT* mutation assays in various test systems involving mammalian cell lines (32,34,35) and rodents (37–39). On the contrary, some studies suggested that AA may not induce gene mutation in *HPRT* test. Tsuda *et al.* (33) and Baum *et al.* (34) reported the negative responses of AA in Chinese hamster V79 cells, independently. In the first experiment, AA did not induce any gene mutation in *Hprt* locus even when V79 cells were treated with 7 mM AA (equal to 500 mg/l) for 24 h. In the second experiment, AA was inactive up to a concentration of 10 mM (equal to 710 mg/L) for 24 h. These results may support the concept of AA by itself not acting as a mutagenic agent at exposure levels to be expected from food ingestion. The concept has also been confirmed by the evidence that AA was non-mutagenic in bacterial gene mutation assays (11,18), in which AA consistently exhibited negative results using different strains of *Salmonella typhimurium*, including TA1535, TA1537, TA1538, TA97, TA98, TA100 and TA102 in the presence or the absence of an exogenous activating system.

However, studies at the *HPRT* gene have exhibited inconsistent mutagenic effects induced by AA in different models. Results obtained in some test models indicate the weakly or moderately positive responses of AA

at *Hprt* locus. In 1988, Knaap *et al.* (18) reported a relatively weak activity of AA in L5178Y mouse lymphoma cells, with the maximum proportion increase of *Hprt* gene mutation for 6-fold at doses where cells exhibited fairly low survival levels. Similarly, mutation study in human promyelocytic leukaemia HL-60 and NB4 cell lines also showed AA exerted a weak mutagenic effect at the highest concentration used in that study after a 6 h treatment without the exogenous activation (36). Although a linear increase in the mutant frequency (MF) with the increasing concentration of AA was found, the significant difference occurred only at the highest concentration of AA (700 mg/L) in HL-60 cells ($p < 0.01$) and NB4 cells ($p < 0.01$), respectively, where the MF was about five times higher than that of the control cultures in both cell lines. More recently, there was increasing evidence which suggested that AA as well as GA, produced increased lymphocyte *Hprt* mutant frequencies (MFs) in rodent animals, including in Big Blue mice, B6C3F1/TK mice and Big Blue rats (37–39). Manjanatha *et al.* investigated the mutagenicity of AA and GA in Big Blue mice (37). Groups of male and female animals were administered 1.4 or 7.0 mM of AA or equimolar doses of GA in drinking water (equivalent to daily received doses of ~ 20 and 100 mg/kg body weight) for 3–4 weeks, then *Hprt* mutation assay was conducted in lymphocytes isolated from spleen. It was found that both AA and GA exposure resulted in significant dose-dependent increases in the *Hprt* MFs, with the high doses producing response 16–25-fold higher than that of the control ($p < 0.01$). Although the result indicated that AA was a mutagen in mice via oral administration, the doses of AA used in the study were higher than those used in cancer bioassays. In the subsequent study, the same research group continued the examination of mutagenicity of AA and GA in Big Blue rats exposed to the doses similar to that used in cancer bioassays (39), 0.7 and 1.4 mM (equivalent to ~ 5 and 10 mg/kg body weight/day) of AA or GA via oral administration for 2 months. Data showed both compounds produced small but significant increases in spleen lymphocyte *Hprt* MF ($p < 0.05$), with the increases having dose-related linear trend ($p < 0.05$ to $p < 0.001$). The MFs in the rats treated with an exposure concentration of 1.4 mM AA (male: $8.0 \pm 4.3 \times 10^{-6}$ and female: $11.6 \pm 2.2 \times 10^{-6}$) were significantly higher than those in the controls ($3.7 \pm 1.7 \times 10^{-6}$ and $7.5 \pm 1.8 \times 10^{-6}$). Altogether, these results indicate a disrupted mutagenicity of AA at the *Hprt* locus in different test models.

According to these results, it can be supposed that the inconsistency in mutagenicity induced by AA should be mainly due to the difference on the metabolic conversion of AA to GA in various models, and this hypothesis was confirmed in a series of studies by comparing the muta-

genic potential between AA and GA in both *in vitro* and *in vivo* tests. In contrast to the negative result of AA obtained at the concentration up to 10 mM in V79 cells without the metabolic activation (34), GA showed a concentration dependent induction of *Hprt* mutations from $800 \mu\text{M}$ upwards (MF: $14 \pm 8 \times 10^{-6}$; solvent control MF: $4 \pm 2 \times 10^{-6}$). The *TK* gene mutation assay also demonstrated that GA is more potent than AA in mutation induction. Koyama *et al.* (31) compared the mutagenicity of both compounds in human lymphoblastoid TK6 cells in the absence of metabolic activation, and found AA was mildly genotoxic in the micronucleus and *TK* assays at high concentrations (> 10 mM), whereas GA was significantly and concentration-dependently genotoxic from 0.5 mM at all endpoints determined, including micronucleus, comet and *TK* assays. Another test in L5178Y mouse lymphoma cells also came to a similar conclusion that GA is much more mutagenic than AA (30). Moreover, *in vivo* experiment in adult Big Blue mice demonstrated the treatment of equimolar doses of AA and GA produced comparable increases in mutant frequencies (37). There is substantial evidence that GA acts as the ultimate genotoxic metabolite in mice, rats and humans (40,41). It interacts with DNA bases, predominantly by forming N7 adducts with guanine and, to a much lower extent, N3 adducts with adenine (42,43). It has been generally believed that GA is a direct-acting mutagen in mammalian cells as well as in bacteria.

Since the significant difference exists between the mutagenic potential of AA and its reactive epoxide metabolite, GA, it should be deduced that more potent mutagenic effects will be exerted in cells or tissues with the elevating capability of metabolic conversion of AA to GA. Therefore these disrupted results of AA above mentioned may be mainly ascribed to the fact that endogenous generation of GA deriving from the activation of CYP2E1 is apparently discrepant in various of systems. AA was reported in 1988 to be mutagenic in *Hprt* gene for the first time in mammalian cell line, L5178Y mouse lymphoma cells (18). This result is similar with that obtained in human leukaemia HL-60 and NB4 cell lines (36). Mouse lymphoma cells have been found to detect the mutagenicity of 2-AAF (44), a promutagen which requires metabolic activation to exert its genotoxic effect, suggesting the residual endogenous metabolic capability in these cells. Likewise, human myelocytic leukaemia cell lines including HL-60 cells, have been reported to have high expression of CYP2E1 (45). In contrast to the negative responses obtained in cells such V79 cells, the distinct mutagenic effects in the *HPRT* gene found in these mouse or human lymphoma cells in the absence of exogenous activation are induced probably through the endogenous metabolism of AA to GA. In mice, about 60% of AA is metabolized by CYP2E1

to GA (46), and mice devoid of CYP2E1 show much reduced levels of male germ cell mutations, micronuclei and GA-DNA adducts compared to their wild-type counterparts, with administration of AA (47–49). These data also support that metabolic activation of AA may be responsible, at least partially, for *HPRT* mutation induction. Certainly, detailed mechanism is worth clarifying in the future.

Comparison of the AA- and GA- induced Mutagenicity between the *HPRT* and *TK* Loci

The genotoxic effects induced by AA and GA also have been estimated with *TK* gene mutation test, which is another widely used mammalian gene mutation assay. Other than the disrupted mutation results from *HPRT* locus, the results obtained at *TK* gene demonstrate a relatively consistent mutagenic potential not only induced by GA, but also induced by AA, which is considered to be a promutagen at *HPRT* locus. As early as 1987, Moore *et al.* (28) reported AA induced a positive mutagenic response in L5178Y mouse lymphoma cells without exogenous activation when tested at 600–650 mg/L, and the highest dose of 850 mg/L resulted in an induced MF of approximately 380×10^{-6} . Subsequently, a series of studies at the *TK* locus demonstrated the consistent results in the same cell line. Knaap *et al.* (18) reported a relatively weak mutagenicity of AA at very low concentration compared with other experiments. Mei *et al.* (30) also found that AA, just as well as GA, increased *Tk* gene MF significantly with the increasing concentrations of compound, although it seems GA is more mutagenic than AA. Likewise, results obtained in human lymphoblastoid TK6 cells indicated a mildly genotoxic effect induced by AA (31). It should be noted that these weakly or mildly positive responses at the *TK* gene were obtained independently of the addition of exogenous metabolic activation systems. Furthermore, Yuan *et al.* (29) detected the genotoxicity of AA in mouse lymphoma cells and found AA exerted a moderate mutagenic effect at the concentration ≥ 300 mg/L, while the assessment of cytotoxicity indicated that AA induced a half decrease in plating efficiency (PE) only at the concentrations ≥ 150 mg/L. The same research group also investigated the AA-induced mutation at the *HPRT* locus, as mentioned above (36), AA exerted a weak mutagenic effect only at the highest concentration of 700 mg/L in HL-60 cells as well as NB4 cells, however, significant toxic effect on PE was already generated at the concentrations ≥ 50 mg/L in HL-60 and ≥ 300 mg/L in NB4 cells. These comparisons suggested that the *TK* gene is more sensitive than the *HPRT* gene to the mutagenicity of AA.

Different from *HPRT* gene mutation assay, which detects only intragenic mutations such as point mutations and small deletions, the genetic endpoints detected by

the *TK* mutation assay are multiple, involving point mutations, small deletions, large-scale chromosomal deletions, and so on (50). Moreover, by calculating the relative frequency of two kinds of colonies, or by analyzing the loss of heterozygosity (LOH) in mutants, *TK* gene mutation test can be used to roughly estimate the cause of genotoxic effect. Davies *et al.* (51) had reported that the difference in sensitivity between these two assays was due to the types of genetic damage induced by agents. If the agents predominantly induce clastogenic effects, the *TK* mutation assay will be more sensitive than the *HPRT* mutation assay. When the agents mainly lead to intragenic mutations, these two assays may have similar sensitivity to detect the mutagenicity of the agents (26,27). As to AA, it is widely believed to be a direct-acting clastogen. AA *in vitro* induces chromosomal aberrations, micronuclei, sister chromatid exchanges and mitotic disturbance with or without the metabolic activation. It also induces positive responses in the *TK* locus *in vivo* and *in vitro* (18,28–31,38). Further analysis of *Tk* mutants in mouse lymphoma cells showed AA induced almost exclusively small-colony mutants, which appear to represent chromosome alterations to that chromosome 11 carrying the *Tk* locus (28). Mei *et al.* (30) revealed all the mutants induced by 16 mM AA, including 48 large colonies and 48 small colonies, lost heterozygosity at the *Tk* locus, confirming the clastogenic mode of action of AA again. On the other hand, Yuan *et al.* (29) found that besides the 78.8% (123/156) of the mutants which showed LOH at the *Tk* locus, there were still 16 LOH-negative mutants which showed base substitutions or an insert mutations. These results, combined with previous results indicating that AA induced substitutions and $-1/+1$ frameshifts in *cII* gene (37,52), and induced weakly positive response at the *HPRT* gene in mammalian cells (18,36), suggest an weak effect of intragenic mutations implicates in AA-induced mutagenesis, besides its predominantly induced large-scale chromosomal deletions.

In contrast, GA is positive in most genotoxicity test and is recognized as a mutagen by producing DNA adducts in target cells. The mutagenic activity induced by GA is much more potent than that of AA not only at the *HPRT* locus, but also at the *TK* locus (30,31,34,37). Moreover, LOH analysis of the *TK* gene showed most GA-induced *TK* mutants were the non-LOH type in human lymphoblastoid TK6 cells (31), suggesting the induction of primarily point mutations for GA. These results confirm the point mutations of GA in bacterial test systems (19), in transgenic mutation test systems (37) and in other mammalian cell test systems such as *HPRT* test (32,34). However, as well as AA, GA was revealed in mouse lymphoma cells to induce LOH in more than 94% of the *Tk* mutants (30), indicating that the mutations mainly resulted from extensive damage to

the chromosome; and that mutagenicity of GA was generated by a clastogenic mode of action rather than a point mutation mode-of-action. This result is entirely different from that obtained in human TK6 cells (31). The difference between these two *TK* gene mutation assays may be due to the different sensitivity to GA treatment and the mutations analyzed from different concentrations (2.2 mM in TK6 cells and 4 mM in mouse lymphoma cells). In addition, p53 gene status is different between these two cell lines. L5178Y/*Tk*^{+/-} mouse lymphoma cells are p53-mutant cell line while TK6 cells have a wild p53 gene. Since studies have proved that LOH and its associated genomic instability strongly depend on p53 status in human lymphoblastoid TK6 cell line and its related cell lines, TK6-E6 and WTK-1 (50), it may be postulated that GA-induced chromosomal mutations in mouse lymphoma cells are most likely associated with the deficiency of functional p53, which is responsible for the elimination and repair of LOH, and a variety of chromosome changes. However, distinct mechanism of GA-induced mutagenicity remains to be investigated in further studies.

Mutation Spectra Analysis of AA and GA at the *HPRT* gene and *TK* gene

Both *HPRT* gene and *TK* gene mutation assays can be used to characterize the molecular mutation spectra, furthermore, to elucidate the mechanisms underlying the genotoxicity induced by various agents, by analyzing the exon deletion, or detecting the DNA sequence of the specific genes in selected mutants. There are consistent results indicating that spontaneous mutation at the *HPRT* locus in some cell types includes mainly point mutations, which can not be distinguished by using multiplex PCR method only (53). However, in the mutation study of human leukaemia HL-60 and NB4 cell line (36), the percentage of exon deletion in AA-induced *HPRT* mutants was much higher than that in spontaneous mutants, and an increase in the proportion of partial deletion was associated with the increase of AA concentration. Moreover, single exon deletion was found to be the predominant mutation of AA. These results suggest that the spectra of spontaneous and AA-induced mutations are distinctly different, and small changes (the nature of the point mutations and single exon deletions) in genetic structure (on the exon level) have something to do with AA-induced mechanisms. However, sequence analysis at the *HPRT* gene is rarely conducted in AA- or GA-induced mutants. Only in Big Blue rats, the mutation spectra induced by AA and GA were analyzed for lymphocyte mutants (39). Sequence analysis differentiated the AA-induced spectrum from the control and GA-induced spectrum, with relatively large difference being an increase in G:C→T:A transversion and frameshifts. However, similar increases in the trans-

version were not observed in the mutation spectrum from GA-treated rats. Because of the small number of *Hprt* mutations identified in the study, any conclusion drawn from the data may be potentially misleading.

Mutation spectrum analyses of the *TK* mutants have provided valuable evidence for understanding the mechanisms of mutagenicity. Koyama *et al.* (31) analyzed the molecular mutation spectra of human *TK* mutants induced by AA or GA with PCR-base LOH analysis, and classified the mutants into three types: non-LOH, hemizygous LOH (hemi-LOH) and homozygous LOH (homo-LOH). In general, hemi-LOH is resulted by deletion and homo-LOH is by inter-allelic homologous recombination. The results showed majority of the AA-induced mutants were hemi-LOH, which is a result of deletion. Because deletions are thought to result from the repair of double strand breaks by non-homologous end-joining, these results supported the hypothesis that predominantly, AA is a clastogen and it causes mainly large-scale chromosome changes including deletion. At the same time, LOH-mapping analysis in chromosome 17q revealed that AA frequently induces intermediate-sized deletions (100–3000kb), and the deletions encompass exons 4 and 7 of the *TK* locus but do not extend to the microsatellite loci of the vicinity. In another study, LOH analysis was conducted using four microsatellites spanning the entire chromosome 11 to determine the types of mutation in mouse lymphoma cells (30). In consistence with the results in human *TK* mutants, AA-induced mouse mutants showed exclusively LOH (100%), confirming the clastogenic mode of action of AA again. However, LOH-mapping analysis revealed these LOH were more likely to extend to microsatellite loci *D11Mit22* and *D11Mit74*, indicating the alteration of DNA larger than half of the chromosome 11. The difference of LOH distribution between human mutants and mouse mutants induced by AA is worth investigating in the future, to better understand the genotoxic mechanism of AA.

Just as above mentioned, molecular mutation spectrum of GA-induced *Hprt* mutants in Big Blue rats is different from that of AA-induced mutants, which showed an increase in G:C→T:A transversion and frameshifts (39). Similarly, statistical analysis of the mutational spectra in *Tk* mutants revealed a significant difference between the types of mutations induced by AA and GA treatment in mouse lymphoma cells ($p = 0.018$) (30). The difference suggests that AA and GA may produce mutations via different mechanisms. GA, the metabolite of AA, acts directly as a mutagen via DNA adduct formation, and GA DNA adduct-specific mutations (i.e., G→T transversions) have also been identified in mammalian cells (54) and animals (37). AA, on the other hand, is predominantly clastogenic along with relatively weak mutagenicity in some sys-

tems. It is generally believed that AA exerts genotoxic effects mainly in two different ways (20). The first is its metabolic conversion to GA, which may be responsible for its mutagenicity at the *HPRT* locus. The second way is that AA can function as a Michael acceptor and form adducts with thiol, hydroxyl or amino groups, and to a lesser extent the nucleophilic centers in DNA (9). Solomon *et al.* (55) demonstrated that AA is slow to react with DNA and only forms adducts under forced chemical conditions and after extended reaction time. However, the Michael-type addition of AA to thiols of glutathione (GSH) occurs most efficiently. The conjugation of AA with GSH can result in depletion of cellular GSH, which is a molecule protecting the cell against endogenous and exogenous oxidants and electrophiles. Others have also reported that AA produces reactive oxygen species that can attack all cellular constituents and induce oxidative DNA damage (14,56). Oxidative stress can cause DNA damage including double-strand breakage, which is repaired through recombinational repair or end rejoining, both of these repair mechanisms could result in LOH. There have been increasing data suggesting the possibility that the latter mechanisms may be responsible for the genotoxicity of AA in some experiments. Puppel *et al.* (57) demonstrated significant inductions of DNA strand breaks with AA treatment in V79 cells that do not express CYP2E1. More recently, Koyama *et al.* (58) reported the equally weak genotoxicity of AA in human lymphoblastoid cell line overexpressing CYP2E1 and its parental cell line, indicating the metabolic activation of AA may not involve to its genotoxic effects in some *in vitro* models. Therefore, the clastogenicity of AA without metabolic activation might result from the relatively increased ROS and/or the impaired oxidative defense system (57).

Summary

In summary, the *in vitro* and *in vivo* mammalian cell gene mutation assays at the *HPRT* gene and *TK* gene have been used to determine the genotoxic potential of AA and its epoxide metabolite GA. In consistence with the results obtained in other mutation tests, mutation changes at the *HPRT* gene and *TK* gene confirm that AA is mainly directly-acting clastogen, causing chromosomal aberrations, AA also produces weakly mutagenic effects at the *HPRT* gene by metabolic conversion of AA to GA. The genotoxic characteristics of GA are distinctly different from AA. GA is a strong mutagen with high reactivity to DNA, inducing predominantly point mutations. Analysis of the molecular mutation spectra in *HPRT* and *TK* mutants identifies the characteristic mutation changes induced by AA or GA respectively, providing clues to elucidate the mechanisms underlying the genotoxicity of AA and GA. Since AA was found in a variety of commonly consumed human foods

and classified as a probable human carcinogen, these investigations on the genotoxicity of AA should help to unravel the mode of action underlying AA-induced carcinogenesis. For the future studies, increasing the knowledge of genotoxic damage in AA-exposed population will help to identify biomarkers used for monitoring of human exposure, to improve the risk evaluation of AA. In addition, the relationship between AA-induced genotoxicity and human genetic polymorphisms is a new field of research and only a limited amount of information is presently available. Genetic polymorphisms in DNA repair, oxidative stress pathway and xenobiotic metabolism may constitute useful biomarkers for the assessment of individual susceptibility to the genotoxic damage induced by AA, and add to the precision of epidemiological studies on cancer associated with AA exposure.

Acknowledgements: Work of the authors is supported by the National Natural Science Foundation of China (No. 30872138), and by the National 973 Project of China (2011CB503705).

Conflict of interest statement: None declared.

References

- 1 Mitka M. Acrylamide-food link questions linger. *JAMA*. 2004; 292: 425.
- 2 Parzefall W. Minireview on the toxicity of dietary acrylamide. *Food Chem Toxicol*. 2008; 46: 1360–4.
- 3 Mottram DS, Wedzicha BL, Dodson AT. Acrylamide is formed in the Maillard reaction. *Nature*. 2002; 419: 448–9.
- 4 Konings EJ, Baars AJ, van Klaveren JD, Spanjer MC, Rensen PM, Hiemstra M, van Kooij JA, Peters PW. Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. *Food Chem Toxicol*. 2003; 41: 1569–79.
- 5 Svensson K, Abramsson L, Becker W, Glynn A, Hellenäs KE, Lind Y, Rosén J. Dietary intake of acrylamide in Sweden. *Food Chem Toxicol*. 2003; 41: 1581–6.
- 6 Erdreich LS, Friedman MA. Epidemiologic evidence for assessing the carcinogenicity of acrylamide. *Regul Toxicol Pharmacol*. 2004; 39: 150–7.
- 7 Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol Biomarkers Prev*. 2007; 16: 2304–13.
- 8 IARC, Acrylamide, IARC Monographs on the Evaluation of Carcinogen Risk to Human: Some Industrial Chemicals, Vol. 60. International Agency for Research on Cancer, Lyon; 1994. p. 389–433.
- 9 Besaratinia A, Pfeifer GP. A review of mechanisms of acrylamide carcinogenicity. *Carcinogenesis*. 2007; 28: 519–28.
- 10 Sumner SC, Fennell TR, Moore TA, Chanas B, Gonzalez

- F, Ghanayem BI. Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. *Chem Res Toxicol*. 1999; 12: 1110–6.
- 11 Dearfield KL, Douglas GR, Ehling UH, Moore MM, Sega GA, Brusick DJ. Acrylamide: a review of its genotoxicity and an assessment of heritable genetic risk. *Mutat Res*. 1995; 330: 71–99.
 - 12 Rice JM. The carcinogenicity of acrylamide. *Mutat Res*. 2005; 580: 3–20.
 - 13 Chico Galdo V, Massart C, Jin L, Vanvooren V, Caillet-Fauquet P, Andry G, Lothaire P, Dequanter D, Friedman M, Van Sande J. Acrylamide, an *in vivo* thyroid carcinogenic agent, induces DNA damage in rat thyroid cell lines and primary cultures. *Mol Cell Endocrinol*. 2006; 257–8: 6–14.
 - 14 Jiang L, Cao J, An Y, Geng C, Qu S, Jiang L, Zhong L. Genotoxicity of acrylamide in human hepatoma G2 (HepG2) cells. *Toxicol in Vitro*. 2007; 21: 1486–92.
 - 15 Cao J, Beisker W, Nüsse M, Adler ID. Flow cytometric detection of micronuclei induced by chemicals in polychromatic erythrocytes of mouse peripheral blood. *Mutagenesis*. 1993; 8: 533–41.
 - 16 Lahdetie J, Suutari A, Sjöblom T. The spermatid micronucleus test with the dissection technique detects the germ cell mutagenicity of acrylamide in rat meiotic cells. *Mutat Res*. 1994; 309: 255–62.
 - 17 Witt KL, Livanos E, Kissling GE, Torous DK, Caspary W, Tice RR, Recio L. Comparison of flow cytometry and microscopy-based methods for measuring micronucleated reticulocyte frequencies in rodents treated with nongenotoxic and genotoxic chemicals. *Mutat Res*. 2008; 649: 101–13.
 - 18 Knaap AG, Kramers PG, Voogd CE, Bergkamp WG, Groot MG, Langebroek PG, Mout HC, van der Stel JJ, Verharen HW. Mutagenic activity of acrylamide in eukaryotic systems but not in bacteria. *Mutagenesis*. 1988; 3: 263–8.
 - 19 Hashimoto K, Tanii H. Mutagenicity of acrylamide and its analogues in *Salmonella typhimurium*. *Mutat Res*. 1985; 158: 129–33.
 - 20 Carere A. Genotoxicity and carcinogenicity of acrylamide: a critical review. *Ann Ist Super Sanita*. 2006; 42: 144–55.
 - 21 Sculley DG, Dawson PA, Emmerson BT, Gordon RB. A review of the molecular basis of hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency. *Hum Genet*. 1992; 90: 195–207.
 - 22 Dobrovolsky VN, Chen T, Heflich RH. Molecular analysis of *in vivo* mutations induced by N-ethyl-N-nitrosourea in the autosomal *Tk* and the X-linked *Hprt* genes of mouse lymphocytes. *Environ Mol Mutagen*. 1999; 34: 30–8.
 - 23 Moore MM, Clive D, Hozier JC, Howard BE, Batson AG, Turner NT, Sawyer J. Analysis of trifluorothymidine-resistant (TFT^r) mutants of L5178Y/TK^{+/−} mouse lymphoma cells. *Mutat Res*. 1985; 151: 161–74.
 - 24 Shao C, Yin M, Deng L, Stambrook PJ, Doetschman T, Tischfield JA. Loss of heterozygosity and point mutation at *Hprt* Locus in T cells and fibroblasts of Pms2^{−/−} mice. *Oncogene*. 2002; 21: 2840–5.
 - 25 Clements J. The mouse lymphoma assay. *Mutat Res*. 2000; 455: 97–110.
 - 26 Applegate ML, Moore MM, Broder CB, Burrell A, Juhn G, Kasweck KL, Lin PF, Wadhams A, Hozier JC. Molecular dissection of mutations at the heterozygous thymidine kinase locus in mouse lymphoma cells. *Proc Natl Acad Sci USA*. 1990; 87: 51–5.
 - 27 Harrington-Brock K, Collard DD, Chen T. Bromate induces loss of heterozygosity in the thymidine kinase gene of L5178Y/Tk^(+/-)-3.7.2C mouse lymphoma cells. *Mutat Res*. 2003; 537: 21–8.
 - 28 Moore MM, Amtower A, Doerr C, Brock KH, Dearfield KL. Mutagenicity and clastogenicity of acrylamide in L5178Y mouse lymphoma cells. *Environ Mutagen*. 1987; 9: 261–7.
 - 29 Yuan J, Liu SX, Liu JY, Cao J. Mutational spectra in the *tk* gene of mouse lymphoma cells induced by acrylamide. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi*. 2005; 23: 125–8. (in Chinese)
 - 30 Mei N, Hu J, Churchwell MI, Guo L, Moore MM, Doerge DR, Chen T. Genotoxic effects of acrylamide and glycidamide in mouse lymphoma cells. *Food Chem Toxicol*. 2008; 46: 628–36.
 - 31 Koyama N, Sakamoto H, Sakuraba M, Koizumi T, Takashima Y, Hayashi M, Matsufuji H, Yamagata K, Masuda S, Kinae N, Honma M. Genotoxicity of acrylamide and glycidamide in human lymphoblastoid TK6 cells. *Mutat Res*. 2006; 603: 151–8.
 - 32 Silviri V, Haglund J, Jenssen D, Golding BT, Ehrenberg L, Törnqvist M. Reaction-kinetic parameters of glycidamide as determinants of mutagenic potency. *Mutat Res*. 2005; 580: 91–101.
 - 33 Tsuda H, Shimizu CS, Taketomi MK, Hasegawa MM, Hamada A, Kawata KM, Inui N. Acrylamide; induction of DNA damage, chromosomal aberrations and cell transformation without gene mutations. *Mutagenesis*. 1993; 8: 23–9.
 - 34 Baum M, Fauth E, Fritzen S, Herrmann A, Mertes P, Merz K, Rudolphi M, Zankl H, Eisenbrand G. Acrylamide and glycidamide: genotoxic effects in V79-cells and human blood. *Mutat Res*. 2005; 580: 61–9.
 - 35 Baum M, Loeppky RN, Thielen S, Eisenbrand G. Genotoxicity of glycidamide in comparison to 3-N-nitroso-oxazolidin-2-one. *J Agric Food Chem*. 2008; 56: 5989–93.
 - 36 Ao L, Liu SX, Yang MS, Fong CC, An H, Cao J. Acrylamide-induced molecular mutation spectra at *HPRT* locus in human promyelocytic leukaemia HL-60 and NB4 cell lines. *Mutagenesis*. 2008; 23: 309–15.
 - 37 Manjanatha MG, Aidoo A, Shelton SD, Bishop ME, McDaniel LP, Lyn-Cook LE, Doerge DR. Genotoxicity of acrylamide and its metabolite glycidamide administered in drinking water to male and female Big Blue mice. *Environ Mol Mutagen*. 2006; 47: 6–17.
 - 38 Von Tungeln LS, Churchwell MI, Doerge DR, Shaddock JG, McGarrity LJ, Heflich RH, da Costa GG, Marques MM, Beland FA. DNA adduct formation and induction of micronuclei and mutations in B6C3F1/Tk mice treated neonatally with acrylamide or glycidamide. *Int J Cancer*.

- 2009; 124: 2006–15.
- 39 Mei N, McDaniel LP, Dobrovolsky VN, Guo X, Shaddock JG, Mittelstaedt RA, Azuma M, Shelton SD, McGarrity LJ, Doerge DR, Heflich RH. The genotoxicity of acrylamide and glycidamide in big blue rats. *Toxicol Sci.* 2010; 115: 412–21.
- 40 Sumner SC, Fennell TR, Moore TA, Chanas B, Gonzalez F, Ghanayem BI. Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. *Chem Res Toxicol.* 1999; 12: 1110–6.
- 41 Abramsson-Zetterberg L. The dose-response relationship at very low doses of acrylamide is linear in the flow cytometer-based mouse micronucleus assay. *Mutat Res.* 2003; 535: 215–22.
- 42 Doerge DR, da Costa GG, McDaniel LP, Churchwell MI, Twaddle NC, Beland FA. DNA adducts derived from administration of acrylamide and glycidamide to mice and rats. *Mutat Res.* 2005; 580: 131–41.
- 43 Gamboa da Costa G, Churchwell MI, Hamilton LP, Von Tungeln LS, Beland FA, Marques MM, Doerge DR. DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal mice. *Chem Res Toxicol.* 2003; 16: 1328–37.
- 44 Amacher DE, Paillet SC, Elliott JA. The metabolism of N-acetyl-2-aminofluorene to a mutagen in L5178Y/TK^{+/−} mouse lymphoma cells. *Mutat Res.* 1981; 89: 311–20.
- 45 Nagai F, Hiyoshi Y, Sugimachi K, Tamura HO. Cytochrome P450 (CYP) expression in human myeloblastic and lymphoid cell lines. *Biol Pharm Bull.* 2002; 25: 383–5.
- 46 Fennell TR, Snyder RW, Krol WL, Sumner SC. Comparison of the hemoglobin adducts formed by administration of N-methylolacrylamide and acrylamide to rats. *Toxicol Sci.* 2003; 71: 164–75.
- 47 Ghanayem BI, Witt KL, El-Hadri L, Hoffer U, Kissling GE, Shelby MD, Bishop JB. Comparison of germ cell mutagenicity in male CYP2E1-null and wild-type mice treated with acrylamide: evidence supporting a glycidamide-mediated effect. *Biol Reprod.* 2005; 72: 157–63.
- 48 Ghanayem BI, Witt KL, Kissling GE, Tice RR, Recio L. Absence of acrylamide-induced genotoxicity in CYP2E1-null mice: evidence consistent with a glycidamide-mediated effect. *Mutat Res.* 2005; 578: 284–97.
- 49 Ghanayem BI, McDaniel LP, Churchwell MI, Twaddle NC, Snyder R, Fennell TR, Doerge DR. Role of CYP2E1 in the epoxidation of acrylamide to glycidamide and formation of DNA and hemoglobin adducts. *Toxicol Sci.* 2005; 88: 311–8.
- 50 Honma M. Generation of loss of heterozygosity and its dependency on p53 status in human lymphoblastoid cells. *Environ Mol Mutagen.* 2005; 45: 162–76.
- 51 Davies MJ, Phillips BJ and Rumsby PC. Molecular analysis of chemically-induced mutations in mammalian cell assays. *Toxicology in Vitro.* 1995; 9: 513–5.
- 52 Besaratinia A, Pfeifer GP. Weak yet distinct mutagenicity of acrylamide in mammalian cells. *J Natl Cancer Inst.* 2003; 95: 889–96.
- 53 Tomita-Mitchell A, Kat AG, Marcelino LA, Li-Sucholeiki XC, Goodluck-Griffith J, Thilly WG. Mismatch repair deficient human cells: spontaneous and MNNG-induced mutational spectra in the *HPRT* gene. *Mutat Res.* 2000; 450: 125–38.
- 54 Besaratinia A, Pfeifer GP. Genotoxicity of acrylamide and glycidamide. *J Natl Cancer Inst.* 2004; 96: 1023–9.
- 55 Solomon JJ, Fedyk J, Mukai F, Segal A. Direct alkylation of 2'-deoxynucleosides and DNA following *in vitro* reaction with acrylamide. *Cancer Res.* 1985; 45: 3465–70.
- 56 Blasiak J, Gloc E, Wozniak K, Czechowska A. Genotoxicity of acrylamide in human lymphocytes. *Chem Biol Interact.* 2004; 149: 137–49.
- 57 Puppel N, Tjaden Z, Fueller F, Marko D. DNA strand breaking capacity of acrylamide and glycidamide in mammalian cells. *Mutat Res.* 2005; 580: 71–80.
- 58 Koyama N, Yasui M, Oda Y, Suzuki S, Satoh T, Suzuki T, Matsuda T, Masuda S, Kinae N, Honma M. Genotoxicity of acrylamide *in vitro*: Acrylamide is not metabolically activated in standard *in vitro* systems. *Environ Mol Mutagen.* 2011; 52: 11–9.