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Mutagenicity and DNA repair of glycidamide-induced adducts in mammalian cells

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Abstract

Glycidamide (GA)-induced mutagenesis in mammalian cells is not very well understood. Here, we investigated mutagenicity and DNA repair of GA-induced adducts utilizing Chinese hamster cell lines deficient in base excision repair (BER), nucleotide excision repair (NER) or homologous recombination (HR) in comparison to parent wild-type cells. We used the DRAG assay in order to map pathways involved in the repair of GA-induced DNA lesions. This assay utilizes the principle that a DNA repair deficient cell line is expected to be affected in growth and/or survival more than a repair proficient cell.

A significant induction of mutations by GA was detected in the *hprt* locus of wild-type cells but not in BER deficient cells. Cells deficient in HR or BER were three or five times, respectively, more sensitive to GA in terms of growth inhibition than were wild-type cells. The results obtained on the rate of incisions in BER and NER suggest that lesions induced by GA are repaired by short patch BER rather than long patch BER or NER. Furthermore, a large proportion of the GA-induced lesions gave rise to strand breaks that are repaired by a mechanism not involving PARP. It is suggested that these strand breaks, which might be the results from alkylation of the backbone phosphate, are misrepaired by HR during replication thereby leading to a clastogenic rather than a mutagenic pathway. The type of lesion responsible for the mutagenic effect of GA cannot be concluded from the results presented in this study.

Keywords: Glycidamide; DNA repair; Homologous recombination; Mutation; Strand breaks

Abbreviations: ADU, alkaline DNA unwinding; AraC, cytosine arabinoside; BER, base excision repair; CHO, Chinese hamster ovary; DMEM, Dulbeccos minimal essential medium with fetal calf serum and antibiotics; DRAG, detection of repairable adducts by growth inhibition; DSB, double strand breaks; EO, ethylene oxide; GA, glycidamide; HEPES, 4-2-hydroxyethyl-1-piperazineethanesulfonic acid; HBSS, Hanksĭ balanced salt solution; HPRT, hypoxanthine–guanine phosphoribosyl transferase; HR, homologous recombination; HU, hydroxyurea; ISQ, 1,5-isoquinolinediol; IC_{50}, inhibiting concentration 50%; IARC, International Agency for Research on Cancer; NER, nucleotide excision repair; PARP, poly(ADP)ribosylation; SDS, sodium dodecyl sulphate; SSB, single strand breaks

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1. Introduction

Glycidamide (GA), the ultimate reactive metabolite to acrylamide (AA) [1,2], has been shown to be mutagenic in bacteria [3] and in a transgenic embryonic mouse fibroblast model [4]. Induction of dominant lethals by AA has been demonstrated in rodents [5], an effect which was suggested to be the result of GA due to metabolic conversion of AA by P-450. These observations are supported by the findings that GA gives rise to micronuclei in both mice and rats [6].

Several DNA adducts have been identified after treatments with GA [7,8]. The most abundant adduct formed after treatments with either AA or GA to mice is N7-(2-carbamoyl-2-hydroxyethyl)guanine (N7-GA-Gua) [7]. In addition, N1-(2-carboxy-2-hydroxyethyl)-2′-deoxyadenosine (N1-GA-dA) and N3-(2-carbamoyl-2-hydroxyethyl)adenine (N3-GA-Ade) have been identified in vitro [8]. Although some data exist on the stability of these adducts, little is known about the mechanism by which they are repaired and their mutagenic potential in mammalian cells.

In the present investigation, we examined the role of different DNA repair pathways in response to DNA damage caused by GA and the implications for acute toxicity and mutagenicity. We applied a DNA repair assay that gives information on incision capacity of short and long patch base excision repair (BER), nucleotide excision repair (NER) and induction of single strand breaks (SSBs). Small base adducts require precise and rapid repair by means of the base excision repair (BER) pathway, or even nucleotide excision repair (NER). We employed a set of Chinese hamster cell lines deficient in different DNA repair pathways developed by Thompson and co-workers [9,10], i.e., the EM9, UV4 and irs1SF cell lines. The DRAG assay [11], monitoring growth inhibition and reduced survival in these cell lines, was used to provide information of the type of DNA repair pathways needed for sustained growth after GA treatments. The alkaline DNA unwinding (ADU) assay, monitoring single strand breaks, was used to investigate induction of SSBs and repair pathways using polymerase inhibitors specific for BER or NER [12]. The *hprt* assay [13] was applied to assess mutagenic potential in an endogenous locus.

2. Materials and methods

2.1. Chemicals

Glycidamide (GA; CAS Nr. 5694-00-8) was synthesized from acrylonitrile according to method B of Payne and Williams [14] and was found to be at least 95% pure [analysis by ^{13}C NMR (acetone-D6, 25 °C): δ 46.94 [CH_2], 49.52 [CH], 171.87 [CO]; m.p. 34–34.5 °C (white crystals from acetone)]. GA was found to be stable during treatment conditions, which was investigated elsewhere [15]. Cytosine arabinoside (AraC), hydroxyurea (HU) and 1,5-isoquinolinediol (ISQ) were obtained from Sigma.

2.2. Cell lines

The AA8, EM9, UV4 and irs1SF cell lines, wild-type and deficient in XRCC1, ERCC1 and XRCC3, respectively, were all obtained from L. Thompson, LLNL Livermore, CA, USA. The cell lines were cultured in minimum essential medium, containing Dulbeccos salt, with the addition of 9% foetal calf serum and penicillin–streptomycin (90 U/mL) (DMEM) at 37 °C and 5% CO_2 in humidified air.

2.3. Treatment conditions

Treatment with GA in the mutagenicity assay was conducted for 1 h in Hank's balanced salt solution with 10 mM HEPES ($HBSS^{++}$), followed by 24 h recovery in DMEM before seeding for cloning and growth for expression of the mutant phenotype. Treatments in the 24-well plates for the repair assays were performed in $HBSS^{++}$ for 30 min and in the 96 well-plates for the DRAG assay in $HBSS^{++}$ for 1 h. Chemical analysis of GA indicated a half-life of 7 h which gives a factor 0.95 in correction of the concentration given to obtain the dose in mM h. This correction was not performed in the ADU experiments since treatments only went on for 0.5 h.

2.4. Survival and mutagenicity assays

The gene encoding hypoxanthine–guanine phosphoribosyl transferase (HPRT) was used for detection of induced mutations according to a slightly modified assay described elsewhere [13].