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Acrylamide and glycidamide: genotoxic effects in V79-cells and human blood

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Abstract

Acrylamide (AA) can be formed in certain foods by heating, predominantly from the precursor asparagine. It is a carcinogen in animal experiments, but the relevance of dietary exposure for humans is still under debate. There is substantial evidence that glycidamide (GA), metabolically formed from AA by Cyp 2E1-mediated epoxidation, acts as ultimate mutagenic agent. We compared the mutagenic potential of AA and GA in V79-cells, using the hprt mutagenicity-test with *N*-methyl-*N*′-nitro-*N*-nitroso-guanidine (MNNG) as positive control. Whereas MNNG showed marked mutagenic effectivity already at 0.5 μM, AA was inactive up to a concentration of 10 mM. In contrast, GA showed a concentration dependent induction of mutations at concentrations of 800 μM and higher. Human blood was used as model system to investigate genotoxic potential in lymphocytes by single cell gel electrophoresis (comet assay) and by measuring the induction of micronuclei (MN) with bleomycin (BL) as positive control. AA did not induce significant genotoxicity or mutagenicity up to 6000 μM. With GA, concentration dependent DNA damage was observed in the dose range of 300–3000 μM after 4 h incubation. Significant MN-induction was not observed with AA (up to 5000 μM) and GA (up to 1000 μM), whereas BL (4 μM) induced significantly enhanced MN frequencies. Thus, in our systems GA appears to exert a rather moderate genotoxic activity.

Keywords: Acrylamide; Glycidamide; Genotoxicity; Mutagenicity; V79 cells; hprt-test; Comet assay; Micronucleus induction

1. Introduction

Acrylamide (AA) is generated by heating certain foodstuffs, depending on heating conditions, type and concentration of precursors [1–4]. AA has been

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reported to be neurotoxic [5] and mutagenic [6–8]. Longterm studies in rodents revealed tumors in some specific organs, such as mesotheliomas in the scrotum, mammary fibroadenomas and adenocarcinomas as well as thyroid follicula adenomas and adenocarcinomas and some other tumors [9–12]. AA was classified as "probably carcinogenic to humans" by a working group of the International Agency for Research on Cancer [13].

Biomarker guided studies using the modified Edman degradation method for the determination of an N-terminal valine in hemoglobin [14,15] demonstrated that high AA exposure might be met under certain working place conditions in the industrial production of polyacrylamide or in a specific situation, for instance due to the use as grouting agent in tunnel construction [16–18]. Smokers are also substantially exposed to AA [19,20]. Total dietary daily AA intake has been estimated to correspond to about 0.5 μg AA per kg body weight (b.w.) on the basis of a normal western diet [21–23].

Several animal studies have demonstrated that AA can cause chromosomal aberrations in vivo in a dose range between 30 and 150 mg/kg b.w. [6,24]. Micronucleus induction in polychromatic erythrocytes from mice at doses lower than 30 mg/kg b.w. was reported by Abramsson-Zetterberg [25]. There is also consistent evidence that AA induces sister chromatid exchanges and chromosomal aberrations in mammalian cells in vitro [7,8]. However, AA has been found inactive in mutagenicity tests with bacteria in the presence or absence of activating systems [26–28]. AA itself does not show substantial reactivity towards DNA. However, under forced chemical conditions and after extended reaction time, adduct formation with DNA bases has been achieved and adducts were chemically characterized [29].

Glycidamide (GA), the ultimate genotoxic metabolite of AA, is generated in mice by CYP4502E1-mediated epoxidation [30]. GA induces mutations in bacteria [31]. After i.p. application of AA (50 mg/kg b.w.) to mice, DNA adducts in different organs have been found, originating from metabolically formed GA. GA adducts with N-7 of guanine and N3 of adenine were major reaction products [32,33]. Intraperitoneally applied GA was found to induce micronuclei (MN) dose dependently (16–61 mg/kg b.w.) in peripheral erythrocytes from mice [34]. Altogether, there is convincing evidence that GA acts as the ultimate genotoxic metabolite of AA.

AA has been found to be conjugated with glutathione [35,36]. In vivo, glutathione adducts follow the degradation pathway to mercapturic acids which are excreted via the urine. In the blood, binding to proteins like hemoglobin (Hb) or albumin and other plasma proteins with nucleophilic centers may contribute to deactivation of AA and GA.

In the present study, we compared mutagenic activity of AA and GA in the hprt-test in V79 cells with *N*-methyl-*N*′-nitro-*N*-nitroso-guanidine (MNNG) as reference mutagen. Furthermore, we used human blood as a model to study concentration dependent interactions with DNA in lymphocytes as an easily accessible substrate to measure genotoxicity. Concentration response relationships of these reactions were investigated. DNA-damage was monitored by single cell gel electrophoresis (comet assay). The potential to induce chromosomal aberrations was investigated by the MN cytokinesis block assay (CBMN).

2. Materials and methods

2.1. Chemicals

AA (for electrophoresis, CAS No. 79-06-1) was obtained from Merck (Darmstadt, Germany). GA (2,3-epoxypropanamide, CAS No. 5694-00-8) was prepared as described by Payne and Williams [37]. Bleomycin (BL) and agarose (low and normal melting point) were obtained from Serva Electrophoresis GmbH (Heidelberg, Germany). MNNG and 6-thioguanine were obtained from Sigma–Aldrich Chemie GmbH (Deisenhofen, Germany). All chemicals were of analytical grade or complied with the standards needed for cell culture experiments.

2.2. hprt-mutation test with V79 cells

V79 cells were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum (FCS), 1 mM sodium pyruvate, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C, 5% CO_2 and 95% saturated