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Induction of sister chromatid exchange by acrylamide and glycidamide in human lymphocytes: Role of polymorphisms in detoxification and DNA-repair genes in the genotoxicity of glycidamide

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ABSTRACT

Acrylamide (AA) is a probable human carcinogen generated in carbohydrate-rich foodstuffs upon heating. Glycidamide (GA), formed *via* epoxidation, presumably mediated by cytochrome P450 2E1, is considered to be the active metabolite that plays a central role in the genotoxicity of AA. The aim of this work was to evaluate the cytogenetic damage induced by AA and GA in cultured human lymphocytes by use of the sister chromatid exchange (SCE) assay. Furthermore, this report addresses the role of individual genetic polymorphisms in key genes involved in detoxification and DNA-repair pathways (BER, NER, HRR and NHEJ) on the induction of SCE by GA. While AA induced the number of SCE/metaphase only slightly, especially for the highest concentration tested (2000 μM), GA markedly induced SCEs in a concentration-dependent manner up to concentrations of 750 μM, leading to an increase in SCEs of up to about 10-fold compared with controls. By combining DNA damage in GA-treated lymphocytes and data on polymorphisms, associations between the induction of SCEs with *GSTP1* (Ile105Val) and *GSTA2* (Glu210Ala) genotypes are suggested.

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1. Introduction

Acrylamide (AA) is a well-known industrial chemical classified as a probable human carcinogen by IARC since 1994 [1]. Until 2002 AA was regarded only as an industrial or occupational genotoxicant. In fact, AA has been used to manufacture polymers, as additives for water treatment, as flow-control agent in oil recovery, in pulp and paper processing, in mining and mineral processing and in laboratory gels. The foremost routes of exposure were considered to be dermal absorption and inhalation of aerosols in the workplace [2,3].

In 2002 it was shown that AA can be formed during heating *via* the Maillard reaction between asparagine and reduced sugars in processed food [4–7]. Moreover, there is evidence that the major contribution for acrylamide-Hb adducts in occupationally non-exposed subjects is originated from acrylamide formation during cooking and food preparation [8]. AA can be found in commonly consumed foods and beverages, such as processed cereals, French fries, potato chips and coffee. Average daily intake of AA was estimated to be about 0.5–1.0 μg/kg bw in adults and up to 2-fold higher in 13-year-old children with a normal western diet [9].

The metabolism of AA occurs *via* conjugation with reduced glutathione (GSH) resulting in the urinary excretion of a mercapturic acid conjugate, or through epoxidation, presumably mediated by cytochrome P450 (CYP2E1), to yield the genotoxic epoxide glycidamide (GA). GA can be metabolized *via* conjugation with GSH or undergo hydrolysis of the epoxide group by epoxide hydrolase (EPHX) to form glyceramide, which is also excreted in urine [6,10,11].

The alpha/beta-unsaturated double bond of AA is responsible for much of its reactivity, being involved in Michael-type reactions. In fact, the beta-carbon of AA can react with nucleophiles [2], leading to formation of protein adducts (*e.g.*, AA-Hb adducts). In addition, the biological activity of AA is also mediated by its metabolite GA. Besides generating protein adducts, GA has a high affinity to DNA, giving rise to DNA-adducts. Conversely, AA has a rather weak capacity to bind DNA [2].

Long-term studies in rodent models have shown that AA is carcinogenic at different organ-sites [6]. However, no consistent evidence of an increased cancer risk was found among workers exposed to AA. Moreover, the association of the increased risk for

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human cancer with dietary consumption of AA is still a matter of discussion [3,6]. While some studies have found significant associations between oral exposure to AA and cancer, others failed to prove such a relationship. For instance, a recent study by Wilson et al. [12] found no association between acrylamide and breast cancer. However, with high acrylamide consumption a greater risk for endometrial and possibly ovarian cancer was observed. Similar findings were reported by Hogervorst et al. [13] whereas Olesen et al. found a positive association with estrogen-positive breast cancer [14]. In view of the conflicting results in the epidemiological studies it is crucial to develop valid toxicological biomarkers to be associated with the information in food-frequency questionnaires (FFQ) in order to improve the assessment of a cancer risk upon oral consumption of AA.

In this context, the primary aim of the present report is to assess the usefulness of sister chromatid exchange (SCE) as a cytogenetic toxicological biomarker in human lymphocytes exposed *in vitro* to AA and GA. Moreover, this study aims to identify possible associations of SCE with biomarkers of susceptibility concerning individual genetic polymorphisms in detoxification and DNA-repair genes. The polymorphisms herein studied comprise genes associated with metabolism, specifically glutathione *S*-transferases (*GSTM1*, *GSTT1*, *GSTP1*, *GSTA2*) and *EPHX1*. Moreover, this study is focused on polymorphisms in DNA-repair genes from the base-excision repair (BER), nucleotide-excision repair (NER), homologous recombination repair (HRR) and non-homologous end-joining repair (NHEJ) pathways, which could be critical in the repair of GA-induced DNA lesions.

2. Materials and methods

2.1. Chemicals

Acrylamide (AA; CAS registry number 79-06-1, ≥99.5% pure) was purchased from Fluka (Buchs, Switzerland) and glycidamide (CAS Registry Number 5694-00-8, >98.5% pure, containing ∼1% AA) was obtained from Toronto Research Chemicals (North York, Ontario, Canada). Fetal calf serum, Ham's F-10 medium, penicillin–streptomycin solution, L-glutamine, phosphate-buffered saline pH 7.4 (PBS), methanol, acetic acid, Hoechst 33258, 5-bromo-2′-deoxyuridine (BrdU), mitomycin C, ethidium bromide and colchicine were purchased from Sigma–Aldrich (St. Louis, MO, USA). Potassium chloride and Giemsa dye was acquired from Merck (Darmstadt, Germany). Phytohemagglutinin (PHA) was purchased from Gibco (Grand Island, NY, USA) and heparin was acquired from B. Braun (Lisbon, Portugal).

2.2. Blood samples collection

Peripheral blood samples were obtained from 13 healthy donors (8 female and 5 male, mean age 28.1 ± 4.3). The samples were collected under sterile conditions by venipuncture in heparinized tubes, coded and analyzed under blind conditions.

All donors were informed about the aim and experimental details of the study and an informed consent was obtained from all participating subjects prior to the start of the study. Each participant completed one standardized questionnaire about health history, lifestyle, alcohol consumption, medication usage, family history of cancer, exposure to indoor/outdoor pollutants, and dietary habits. All individuals were non-smokers. Ethical approval for this study was obtained from the institutional Ethical Board of the Faculty of Medical Sciences.

2.3. Lymphocyte culture

The lymphocyte cultures were set up by adding 0.5 ml of whole blood to 4.5 ml of Ham's F-10 medium supplemented with 24% fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, 1% L-glutamine, and 50 IU/ml heparin. Lymphocytes were stimulated with 1.5% (v/v) of PHA and incubated at 37 °C for 72 h in an atmosphere containing 5% CO_2.

2.4. Sister chromatid exchange assay

Firstly, different concentrations of AA and GA up to 2000 μM (dissolved in PBS, pH 7.4) were evaluated in order to characterize the dose–response pattern of both chemicals. For this purpose 24-h cultures of lymphocytes from two donors were treated with AA or GA and two independent experiments were performed for each donor. From the dose–response curve of GA, the concentration of 250 μM was chosen to be further assayed in all the donors (two replicate cultures).

For both studies, after 46 h of incubation with AA or GA in the presence of BrdU (final concentration, 10 μM), lymphocytes were washed with fresh culture medium and colchicine (0.6 μg/ml) was added. The lymphocytes were then incubated for a further 1.5 h. Differential staining of BrdU-substituted sister chromatids was performed according to the fluorescence-plus-Giemsa method [15]. Briefly, the slides were stained for 12 min with the fluorescent dye Hoechst 33258 (10 μg/ml) in 2% KCl (w/v), exposed to UV (254 nm) for ∼9 min, and then stained with 4% Giemsa ([v/v] in 10 mM phosphate buffer, pH 6.8) for 10 min. The frequency of SCE in each metaphase (SCE/metaphase) was scored in 30 second-division metaphases, whenever possible, for each concentration in each experiment. Mitomycin C (0.75 μM) was used as a positive control.

2.5. Mitotic index

The mitotic index (MI) was used as a measure of the cell proliferation. This index can be defined as MI = (no. of cells in division/total no. of cells) × 100 [16]. To determine this index, 1000 lymphocytes were scored for each experiment and the number of metaphases recorded.

2.6. DNA extraction

Genomic DNA was obtained from 250 μl of whole blood using a commercially available kit, according to the manufacturer's instructions (QIAamp DNA extraction kit; Qiagen, Hilden, Germany). Each DNA sample was stored at −20 °C until analysis.

2.7. Genotyping

2.7.1. Detoxification pathways

Genotyping of *GSTM1* and *GSTT1* for gene deletions was carried out by a multiplex PCR as described by Lin et al. [17] with minor modifications described in Costa et al. [18]. After electrophoretic separation the amplified products were visualized in 2% agarose gel stained with ethidium bromide (2.0 μg/ml).

The genotyping of *GSTP1* Ile105Val (rs1695), *EPHX1* Tyr113His (rs1051740) and His139Arg (rs2234922) and *GSTA2* Glu210Ala (rs6577) was conducted with the polymerase chain reaction and restriction fragment length polymorphisms (PCR-RFLP). *GSTP1* Ile105Val genotyping was performed according to Gaspar et al. [19], *EPHX1* Tyr113His and His139Arg polymorphisms were determined as described by Teixeira et al. [20] and *GSTA2* Glu210Ala polymorphisms were determined according to published procedures [21,22] with minor modifications. For the *EPHX1* genotypes in codons 113 and 139, individuals were classified according to the expected activity on the basis of their genotypes as: low activity: His/His–His/His; His/His–His/Arg; Tyr/His–His/His; His/His–Arg/Arg; medium activity: Tyr/Tyr–His/His; Tyr/His–His/Arg; Tyr/His–Arg/Arg; high activity: Tyr/Tyr–Arg/Arg; Tyr/Tyr–His/Arg [21,23].

2.7.2. DNA-repair pathways

The genotyping of *XRCC2* Arg188His (rs3218536), *XRCC3* Thr241Met (rs861539) and *XPC* Lys939Gln (rs2228001) and Ala499Val (rs2228000) polymorphisms was performed by means of PCR-RFLP. *XRCC2* Arg188His and *XRCC3* Thr241Met were performed according to Bastos et al. [24] and *XPC* Lys939Gln and Ala499Val were carried out as described by Pingarilho et al. [25] with minor modifications.

APEX Asp148Glu (rs1130409; C_8921503_10), *ERCC1* Gln504Lys (rs3212986; C_2532948_10), *ERCC2* Lys751Gln (rs13181; C_3145033_10), *ERCC4* Arg415Gln (rs1800067; C_3285104_10), *ERCC5* Cys529Ser (rs2227869; C_15956775_10) and His1104Asp (rs17655; C_1891743_10), *ERCC6* Gln1413Arg (rs2228529; C_16171343_10) and Arg1230Pro (rs4253211; C_25762749_10), *GSTA2* Pro110Ser (rs2234951; C_12027714_50) and Ser112Thr (rs2180314; C_22275149_30), *Ku80* Ex21-238G → A (rs2440; C_3231046_20); Ex21 + 338T → C (rs1051677; C_8838367_1_), Ex21 − 352C → A (rs6941; C_8838374_10), Ex21 + 466A → G (rs1051685; C_8838368_1_), *LIG4* Thr9Ile (rs1805388; C_11427969_20), *MUTYH* Gln335His (rs3219489; C_27504565), *NBS1* Glu185Gln (rs1805794; C_26470398_10), *OGG1* Ser326Cys (rs1052133; C_3095552_1_), *PARP1* Val762Ala (rs1136410; C_1515368_1_), *PARP4* Gly1280Arg (rs13428; C_8700143_10) and Pro1328Thr (rs1050112; C_8700142_10), *RAD23B* Ala249Val (rs1805329; C_11493966_10), *RAD51* 5′UTR (rs1801321; C_7482700_10), *XRCC1* Arg194Trp (rs1799782; C_11463404_10) and Gln399Arg (rs25487; C_622564_10) and *XRCC4* It7G > A (rs1805377; C_11685997_10) and Thr134Ile (rs28360135; C25618660_10) polymorphisms were genotyped by Real-Time PCR (AB7300), using TaqMan SNP Genotyping Assays from Applied Biosystems, according to the manufacturer's instructions and previous studies from our group [22,24,26,27]. DNA samples were quantified with PicoGreen dsDNA Quantification Reagent (Molecular Probes, Eugene, OR, USA) according to the manufacturer's recommendations.

Genotype determinations were carried out twice (all samples for multiplex and PCR-RFLP and 20% of samples for Real-Time PCR) in independent experiments and all the inconclusive samples were reanalyzed.

2.8. Statistical analysis

For the concentration–response curves of AA and GA, regression analyses were performed using the Graphpad Prism v.5 software. The non-parametric

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