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Genotoxicity of glycidamide in comparison to (±)-anti-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide and α-acetoxy-*N*-nitroso-diethanolamine in human blood and in mammalian V79-cells

Silke Thielen[1], Matthias Baum[1], Michelle Hoffmann[1], Richard N. Loeppky[2] and Gerhard Eisenbrand[1]

[1]Department of Food Chemistry and Environmental Toxicology, University of Kaiserslautern, Kaiserslautern, Germany
[2]Department of Chemistry, University of Missouri, Columbia, MO, USA

Genotoxic activity of glycidamide (GA) was investigated in comparison to that of the known carcinogens (±)-anti-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide ((±)-BPDE) and α-acetoxy-*N*-nitroso-diethanolamine (α-A-NDELA), using the hypoxanthine-phosphoribosyl-transferase (*hPRT*) gene mutation assay with V79 mammalian cells and modified alkaline single cell gel electrophoresis (alkaline comet assay with and without treatment of cells with formamido-pyrimidine-DNA-glycosylase (FPG)) in lymphocytes from human whole blood. As shown earlier, GA induced significant DNA damage in lymphocytes from treated whole blood at ≥300 μM (4 h) (Baum *et al.*, *Mutat. Res.* 2005, *580*, 61–69). In the present study, using the alkaline comet assay with FPG treatment, increased formation of DNA strand breaks was observed in lymphocytes treated with GA (10 μM; 4 h). α-A-NDELA and (±)-BPDE were genotoxic at 10–30 μM (1 h). Genotoxic activity of these compounds was not enhanced after FPG treatment. FPG treatment thus offers an enhanced sensitivity of DNA damage detection for genotoxic compounds with preference for N^7- resp. N^3-purine alkylation. In the *hPRT* assay with V79 cells, mutagenic activity of (±)-BPDE became significant at ≥3 μM (24 h). For α-A-NDELA significant activity was observed at ≥10 μM (24 h). As previously observed, GA was considerably less effective, inducing significant mutagenicity roughly at about 80–300-fold higher concentrations (800 μM; 24 h) (Baum *et al.*, *Mutat. Res.* 2005, *580*, 61 – 69).

Keywords: α-acetoxy-NDELA / (±)-BPDE / Comet assay / Glycidamide / *hPRT* gene mutation assay

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1 Introduction

In the mammalian organism, glycidamide (GA) is metabolically formed primarily by CYPIIE1-mediated epoxidation from acrylamide (AA). AA is generated in heated foodstuffs in substantial amounts depending on the heating conditions and the concentration of precursors [2]. AA itself is not or only marginally genotoxic and does not show substantial reactivity towards DNA [1–3]. Its carcinogenicity is ascribed to GA, interacting effectively with DNA bases, predominantly forming N^7-adducts with guanine and N^3-adducts with adenine [3]. Using worst case assumptions of dietary AA exposure a life-time cancer risk in the range of 0.3×10^{-3} to 5.2×10^{-3} was derived from animal experiments. Lowering the AA exposure from food appears therefore of high priority for consumer protection [2].

In our previous findings GA induced DNA damage in human blood lymphocytes dose-dependently at ≥300 μM (4 h), while AA was inactive under these conditions [1].

In the present study we compared the genotoxic activity of GA with that of other activated forms of known carcino-

Correspondence: Professor Gerhard Eisenbrand, Department of Food Chemistry and Environmental Toxicology, University of Kaiserslautern, D-67663 Kaiserslautern, Germany
E-mail: eisenbra@rhrk.uni-kl.de
Fax: +49-631-205-3085

Abbreviations: AA, acrylamide; **α-A-NDELA**, α-acetoxy-*N*-nitroso-diethanolamine; **(±)-BPDE**, (±)-anti-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide; **CE**, cloning efficiency; **FPG**, formamido-pyrimidine-DNA-glycosylase; **GA**, glycidamide; ***hPRT***, hypoxanthine-phosphoribosyl-transferase; **MF**, mutant frequencies; **NER**, nucleotide excision repair; **NHMOR**, *N*-nitroso-2-hydroxymorpholine; **SCGE**, single cell gel electrophoresis; **SSB**, single-strand breaks; **TI**, tail intensity

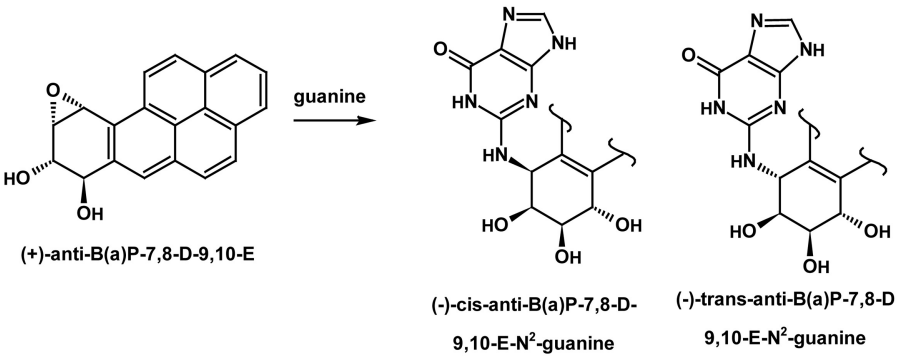

Figure 1. N^2-Guanine adducts of (+)-anti-BPDE.

gens, the benzo[*a*]pyrene metabolite (±)-anti-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide ((±)-BPDE) and α-acetoxy-*N*-nitroso-diethanolamine (α-A-NDELA), an activated form of the procarcinogen NDELA, for which the mechanisms of action are well characterized. We used the modified alkaline single cell gel electrophoresis (SCGE) with additional formamido-pyrimidine-DNA-glycosylase (FPG) treatment in human whole blood and the hypoxanthine-phosphoribosyl-transferase (*hPRT*) gene mutation assay in V79 cells.

The highly reactive bay region diol epoxide BPDE is metabolically formed by CYP450 monooxygenases from the polycyclic aromatic hydrocarbon (PAH) benzo[*a*]pyrene [4]. The primarily formed isomer (+)-anti-BPDE exhibits the highest mutagenicity as well as the predominant capability of adduct formation in DNA [5].

BPDE interacts with DNA bases, forming covalent bulky adducts [5, 6]. The exocyclic N^2 amino group in DNA is the primary target for adduct formation, yielding stable DNA-adducts that have either *trans*- or *cis*-stereoisomeric configurations (Fig. 1) [5, 7, 8]. In minor amounts N^7-adducts of guanine, N^6 of adenine and N^3 of cytosine are formed [9].

BPDE-DNA-adducts at N^2 of guanine are removed by nucleotide excision repair (NER) as observed in *Escherichia coli* and in mammals [10, 11]. The formation of apurinic sites was also observed, probably due to the depurination of N^7-adducts [9]. These modifications should be effectively repaired by the base excision repair (BER) pathway. Generally the BER pathway is fast and efficient, while the NER pathway is relatively slow, enhancing the risk of unrepaired lesions to eventually persist in DNA [9]. Hence, damaged nucleotides may result in mutation(s) during replication [12].

BPDE was found to induce G >T transversions in bacteria and bp substitutions in mammalian cells *in vitro*. Additionally, bulky adducts of BPDE to DNA bases can induce frameshift mutations, deletions and strand breaks in mammalian cells [5, 13].

In comparison, we investigated the genotoxic activity of the nitrosamine α-A-NDELA, a stabilized α-hydroxy-derivative of the potent carcinogen NDELA. NDELA itself undergoes both, CYP450-mediated α-hydroxylation as well as β-oxidation to the cyclic hemiacetal metabolite *N*-nitroso-2-hydroxymorpholine (NHMOR) [14]. NHMOR was found to be a direct bacterial mutagen as well as an inducer of single-strand breaks (SSB) in primary rat hepatocytes and in rat liver [15, 16]. Further oxidation of NHMOR leads to glyoxal that interacts with guanine, forming a cyclic 6,7-dihydroxy-1,N^2-ethano-dG (glyoxal-dG) adduct [14].

The unstable α-hydroxy-NDELA is generated by CYPIIE1-mediated α-hydroxylation of NDELA or, alternatively from α-A-NDELA by hydrolysis of its acetate ester-bond [17] (Fig. 2). This hydroxy-intermediate decomposes to glycolaldehyde and a highly reactive 2-hydroxyethyldiazonium-ion [14]. The latter hydroxyethylates nucleophilic centres of DNA, such as N^7 [18] and O^6 of guanine [14, 15]. Moreover, preferential alkylation of phosphodiester bonds at the DNA-sugar-phosphate-backbone results in phosphotriester formation as a predominant lesion. However, whereas alkyl-phosphotriesters are highly stable in DNA, the corresponding 2-hydroxyethyl-analogues are unstable, creating DNA SSB by rapid spontaneous decomposition [19–21].

Dose-response studies have shown that NDELA is a potent carcinogen in rat and Syrian golden hamster [22, 23]. NDELA was found to become mutagenic to *Salmonella typhimurium* T98 and T100 when activated by alcohol dehydrogenase [24]. After metabolic activation it has been shown to induce DNA-SSB *in vitro* in the human lymphoblastoid cell line Namalva [15]. After oral NDELA-application to rats, DNA strand break formation was observed in the liver [15, 25, 26]. Moreover, using P450-2E1-transfected V79 cells, NDELA was found to be cytotoxic and mutagenic, reflecting the involvement of P450-2E1 in its bioactivation [27]. In human lymphocytes *in vitro* NDELA was found to induce chromosome aberrations, sister chromatid exchanges (SCE) and micronuclei dose-dependently with and without an activating system [28, 29].

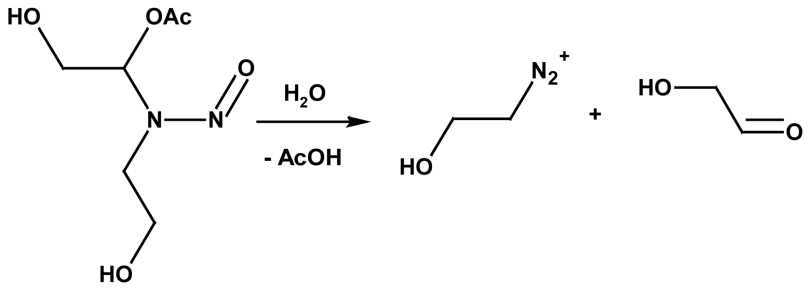

Figure 2. Deesterification of α-A-NDELA results in the release of 2-hydroxyethyl-diazonium-ion and glycolaldehyde.

The comet assay in various modifications is a well established test system to detect DNA strand breaks [30]. It has not been widely used yet in human whole blood as an *ex vivo* system [31, 32]. In contrast to isolated peripheral blood lymphocytes, whole blood more closely mimics the situation after absorption from the gut, providing the relevant target for genotoxicity, the white blood cell DNA, in the presence of noncritical blood constituents of high reactivity towards AA/GA, such as albumin, glutathione and hemoglobin. Additional treatment of lymphocyte-DNA with the DNA repair enzyme formamido-pyrimidine-DNA-glycosylase (FPG) leads to an enhancement of strand breaks at positions where FPG recognizes apurinic and apyrimidinic sites as well as ring-opened pyrimidines (formamido-pyrimidines) and oxidized purines [33].

In the *hPRT* gene mutation assay the mutagenic potential of (±)-BPDE, α-A-NDELA and GA [1] was investigated in comparison, detecting genotoxic events which are not repaired and result in a mutation after several days duration.

2 Materials and methods

2.1 Chemicals

FPG was provided by A. R. Collins, University of Oslo, Norway; (±)-BPDE was synthesized by Dr. A. Seidel, Biochemical Institute for Environmental Carcinogens, Grosshansdorf, Germany and was kindly provided by P. Steinberg, University of Potsdam, Germany. GA (2, 3-epoxypropanamide) was prepared according to Payne & Williams [34]. 6-Thioguanine was obtained from Sigma-Aldrich Chemie (Deisenhofen, Germany).

All chemicals were of analytical grade or complied with the standards needed for cell culture experiments.

2.2 Alkaline SCGE, alkaline comet assay

Blood samples were taken from three male healthy donors by venipuncture using heparinized blood tubes (Sarstedt AG, Nümbrecht, Germany). Individual blood samples of each donor (990 µL) were incubated for 1, 2 and 4 h with substance solution (10 µL, DMSO) at 37°C.

Alkaline SCGE was performed according to Singh *et al.* [35] with slight modifications. Whole blood (6 µL) was mixed with low-melting point agarose (65 µL) and distributed onto a slide precoated with a layer of normal-melting point agarose. The agarose layer was covered with a coverslip and kept at 4°C to allow solidification of agarose. After removing the coverslip, slides were immersed in lysis solution for 1 h at 4°C. After three washings with enzyme buffer, cells were incubated for 30 min at 37°C with either 50 µL of enzyme buffer or FPG dissolved in enzyme-buffer as described [36]. After DNA unwinding (pH >13, 20 min, 4°C), horizontal gel electrophoresis (BioRad Sub Cell GT) was performed at 4°C for 20 min (25 V, 300 mA). After washing the slides three times with 0.4 M Tris, pH 7.5, they were stained with ethidium bromide. Microscopic analysis was done subsequently with a Zeiss Axioskop 2, equipped with filter set 15 (excitation, BP 546/12; emission, LP 590). Slides were analysed by computerized image analysis (Perspective Instruments, Haverhill, UK), scoring 2×50 cells *per* slide (2 gels/slide). DNA migration is expressed as mean tail intensity (TI%). Relative TI is defined as relative rate of comet head intensity to TI [37]. Microscopic analysis was done with a Zeiss Axioskop 20, equipped with a filter set 15 (excitation BP 546/12; emission, LP 590). TI of cells was quantified by computer-assisted microscopy using Comet II®-software.

2.3 *hPRT* gene mutation assay with V79 mammalian cells

Cells were maintained in DMEM, supplemented with 10% FCS, 1 mM sodium pyruvate, 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C, 5% CO_2 and 95% saturated atmospheric humidity.

The *hPRT* assay was performed according to Bradley *et al.* [38], with slight modifications. Cells (1×10^6) were seeded into 75 cm^2 cell culture flasks with 15 mL of DMEM medium. After 24 h, medium was replaced by medium containing the test compounds, predissolved in DMSO (final concentration 1%). After 24 h incubation, medium was

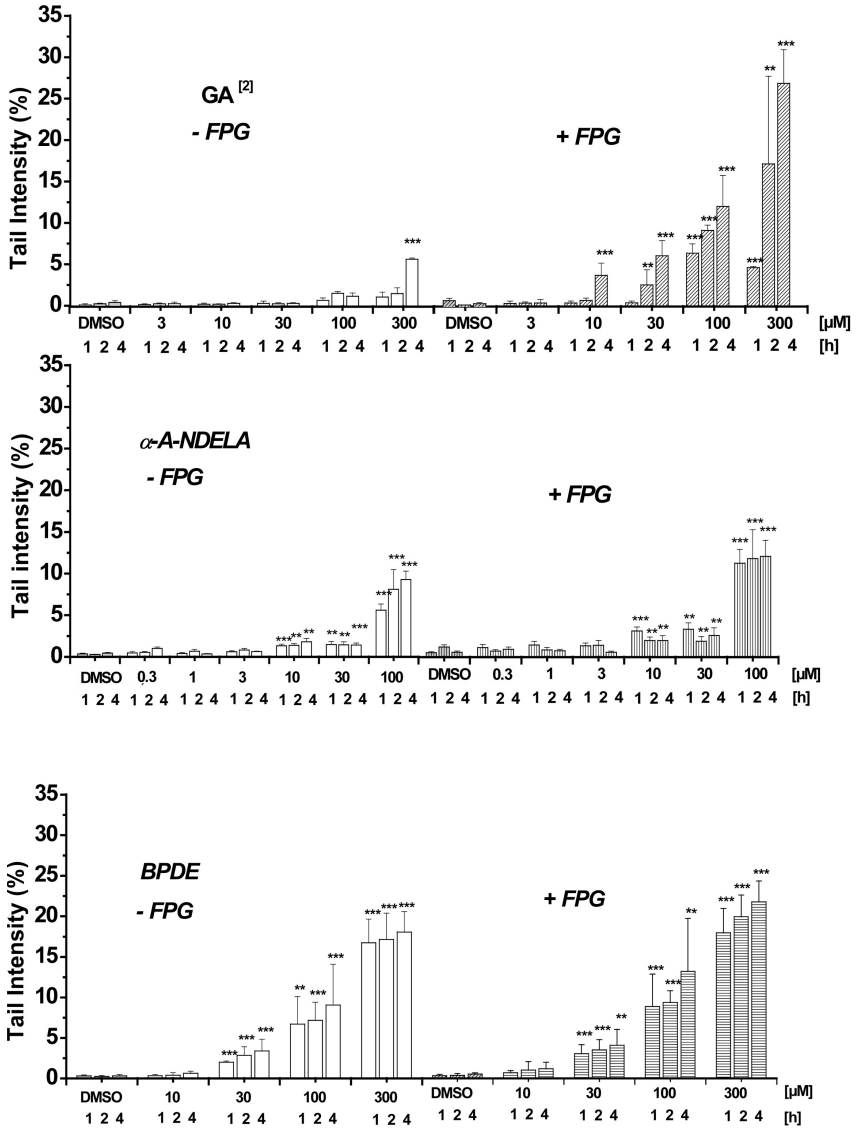

Figure 3. DNA damage in lymphocytes after incubation of human blood with GA [1], α-A-NDELA and (±)-BPDE (1–4 h) with and without FPG treatment (control: DMSO; *p<0.5; **p<0.05; ***p<0.005 (one-sided paired Student's t-test); values represent means and SD of three experiments (three different donors); n = 3).

exchanged against DMEM. Mutations were expressed during a period of 5 days, including two subculturing steps. Mutant frequencies (MF: mutants/10^6 cells) were determined in triplicate and cloning efficiencies (CEs) were scored in duplicate (240 cells *per* plate) as described [39]. MF was calculated from the number of detected mutants multiplied with a correction factor F derived from CE (F = 240/number of scored colonies; MF = mutants × F). Mutagenic potential of substances was compared by calculating D_{3C}-values, reflecting the concentration that induced a three times increase in mutagenicity compared to the solvent control.

Cytotoxicity was monitored in cell suspension by determination of trypan blue exclusion as described elsewhere [1, 40].

2.4 Statistics

Data were statistically analysed using one-sided paired Student's t-test and two-way ANOVA. Normal distribution was ascertained by Anderson-Darling-test and standardized normal distribution (Gaussian).

3 Results

3.1 Induction of DNA strand breaks (SCGE)

In the alkaline comet assay in human whole blood with and without FPG treatment of lymphocyte-DNA all compounds tested were time- and concentration-dependently active. No significant different responses of donors towards compounds were observed within the separate assays.

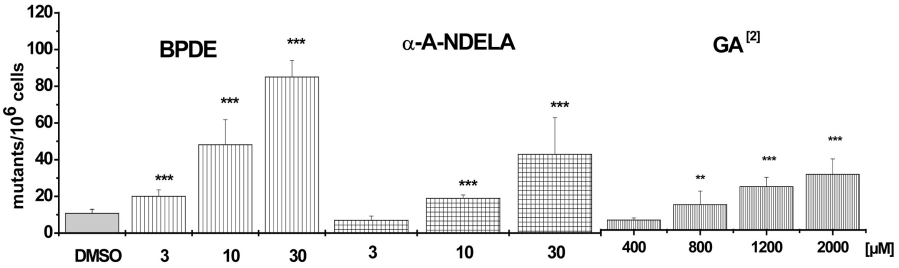

Figure 4. Induction of *hPRT* mutations in V79 cells by (±)-BPDE, α-A-NDELA and GA [1] (incubation time: 24 h; control: DMSO) (mutations are selected by treatment of cells with 6-thioguanine; $^{**}p<0.05$; $^{***}p<0.005$ (one-sided paired Student's *t*-test); values represent means and SD of three experiments; $n = 3$).

Without FPG treatment, (±)-BPDE and α-A-NDELA induced DNA damage at ≥30 and 10 µM (1 h) (Fig. 3). As previously reported, DNA damage in whole blood lymphocytes induced by GA (Comet assay without FPG treatment) became detectable at a ten-fold higher concentration only (300 µM; 4 h) [1]. After additional FPG treatment of lymphocyte-DNA, however, a significant increase (two-way ANOVA) of DNA strand breaks, compared to the comet assay without FPG treatment, was observed with GA (10 µM; 4 h). In contrast, DNA damage induced by (±)-BPDE and α-A-NDELA was not enhanced after FPG treatment (Fig. 3).

3.2 Induction of *hPRT* mutations in V79-cells

In the *hPRT* gene mutation assay, the ability of (±)-BPDE and α-A-NDELA to induce gene mutations at the *hPRT* locus in V79 Chinese hamster lung fibroblasts was investigated in comparison to our previous results received for GA [1].

(±)-BPDE significantly induced *hPRT* mutations, beginning at 3 µM (MF: 20 ± 4; D_{3C}: 7 µM). α-A-NDELA was significantly active at =10 µM (MF: 19 ± 2; D_{3C}: 9 µM) (Fig. 4).

Mutations induced by GA became detectable at 800 µM. Thus, (±)-BPDE and α-A-NDELA showed mutagenic activity in V79 cells in a lower concentration range (80–300-fold) than GA (800 µM; D_{3C}: 670 µM) [1] (Fig. 4).

Under our test conditions (±)-BPDE and α-A-NDELA had no influence on cell viability. As demonstrated earlier, GA was cytotoxic at 800 µM and higher [1]. As a further control, cloning efficiency was monitored after day 5 and was not found to be influenced.

4 Discussion

As demonstrated in our previous studies, GA induced DNA damage in human whole blood in a concentration-dependent manner with significantly enhanced effects beginning at 300 µM (4 h) [1]. Additional treatment of lymphocyte-DNA with FPG allowed DNA strand breaks to become detectable at concentrations as low as 10 µM (4 h). GA primarily forms N^7-adducts of guanine. These adducts yield 5-*N*-alkyl-2,6-diamino-4-hydroxyformamidopyrimidine (alkyl-FAPy-G lesions) after ring-opening or alternatively may generate apurinic sites (AP sites) *via* depurination [41]. Depurination is the favoured reaction under physiological conditions. Under alkaline comet assay conditions, AP sites are rapidly converted into strand breaks [37]. Alkyl-FAPy-G lesions are stable under physiological conditions. They are, however, efficiently removed by specific DNA repair enzymes such as FPG [41]. Thus, GA-related strand breaks observed without FPG treatment may be ascribed, amongst others, to AP sites. However, a certain proportion of AP sites remain undetected under the alkaline comet conditions, as they are not converted into DNA strand breaks [37]. FPG recognizes AP sites as well as FAPy-G lesions, converting these lesions into additional DNA strand breaks. It can therefore be concluded that the enhancement of DNA strand breaks observed after FPG treatment is in line with the premise that GA induces both kinds of lesions.

(±)-BPDE is genotoxic at ≥30 µM in the comet assay in human whole blood. Activity was not enhanced after FPG treatment. Mutagenic potential in the *hPRT* assay became significant at ≥3 µM (24 h). Incubating the human cell line MRC5CV1 with (±)-BPDE, Hanelt *et al.* [13] found mutagenic potential as well as DNA strand breaks in the comet assay after 2 h in a similar concentration range. Investigating the induction of 8-azaguanine resistant colonies by (+)-anti-BPDE, Wood *et al.* [42] detected mutants at ten-fold lower concentrations, reflecting the fact that (+)-BPDE shows the highest mutagenicity as well as the predominant capability of adduct formation in DNA [5]. It has been demonstrated that BPDE interacts covalently with DNA, preferentially binding to exocyclic amino-groups of deoxyguanosine (dG) or deoxyadenosine (dA). This interaction yields predominantly N^2-dG bulky adducts (80–90% of the total adducts induced) [43]. These adducts are stable and are not recognized by FPG. As reported earlier, DNA strand breaks induced by (±)-BPDE in the comet assay without FPG treatment might be due to formation of alkali-labile sites [44] and repair processes, especially excision repair [45] as a consequence of DNA damage. Adducts with N^6 of adenine and N^3 of cytosine are formed to a lesser extent, as

well as adducts with N^7 of guanine [8]. As mentioned above, AP sites which are a result of unstable N^7-guanine-adducts should become visible as DNA strand breaks without FPG treatment to some extend as a consequence of alkaline comet assay conditions. The remaining AP sites would have to be recognized by FPG. We do not see a significant difference between DNA damage with and without FPG treatment, obviously reflecting a low level of N^7-adduct adduct formation.

Under our conditions tested, α-A-NDELA significantly induced DNA damage in human whole blood lymphocytes and *hPRT* mutations in V79 cells at ≥10 µM. α-A-NDELA decays forming a hydroxyethylating intermediate that reacts with DNA bases like guanine by forming N^7- [18] or O^6-hydroxyethyl-adducts [14, 17, 25]. It might, however, also interact to a substantial proportion with the sugar phosphate backbone to generate 2-hydroxyethyl-phosphotriesters similar to other 2-hydroxyethylating substances, creating DNA strand breaks by rapid spontaneous decomposition [19–21]. O^6-hydroxyethyl-adducts are not recognized by FPG.

In summary, depending on the type of genotoxic agent applied, additional FPG treatment appears to offer a promising tool to enhance sensitivity of detection and/or to allow conclusions on the putative underlying mechanism.

The *hPRT* gene mutation assay in V79 cells responds at similar sensitivity towards (±)-BPDE and α-A-NDELA, whereas its response to GA is lower by orders of magnitude [1]. Since the *hPRT* assay encompasses about ten cell division rounds after mutagen challenge, lesions induced by GA might be more effectively repaired or are intrinsically less promutagenic. Further studies on the rate of repair and the types of mutations induced by GA are under investigation.

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5 References

[1] Baum, M., Fauth, E., Fritzen, S., Herrmann, A. *et al.*, *Mutat. Res.* 2005, *580*, 61 – 69.

[2] Dybing, E., Farmer, P. B., Andersen, M., Fennell, T. R. *et al.*, *Food Chem. Toxicol.* 2005, *43*, 365 – 410.

[3] Da Costa, G., Churchwell, M. I., Hamilton, P., Tungeln von, L. S. *et al.*, *Chem. Res. Toxicol.* 2003, *16*, 1328 – 1337.

[4] Conney, A. H., Chang, R. L., Jerina, D. M., Wei, S. J., *Drug Metab. Rev.* 1994, *26*, 125 – 163.

[5] Opinion of the Scientific Committee on Food on the risk to human health of Polycyclic Aromatic Hydrocarbons in food, European Commission 2002 (expressed on 3 July, 2002).

[6] Peltonen, K., Dipple, A., *J. Occup. Environ. Med.* 1995, *37*, 52 – 58.

[7] Graslund, A., Jernstrom, B., *Q. Rev. Biophys.* 1989, *22*, 1 – 37.

[8] Grollman, A. P., Shibutani, S., *IARC Sci. Publ.* 1994, *125*, 385 – 397.

[9] Braithwaite, E., Wu, X., Wang, Z., *Carcinogenesis* 1998, *19*, 1239 – 1246.

[10] van Houten, B., *Microbiol. Rev.* 1990, *54*, 18 – 51.

[11] Gunz, D., Hess, M. T., Naegeli, H., *J. Biol. Chem*. 1996, *271*, 25089 – 25098.

[12] Suzuki, N., Ohashi, E., Kolbanovskiy, A., Geacintov, N. E. *et al.*, *Biochemistry* 2002, *41*, 6100 – 6106.

[13] Hanelt, S., Helbig, R., Hartmann, A., Lang, M. *et al.*, *Mutat. Res.* 1997, *390*, 179 – 188.

[14] Dennehy, M. K., Loeppky, R. N., *Chem. Res. Toxicol.* 2005, *18*, 556 – 565.

[15] Denkel, E., Sterzel, W., Eisenbrand, G., *IARC Sci. Publ.* 1987, *84*, 83 – 86.

[16] Denkel, E., Pool, B. L., Schlehofer, J. R., Eisenbrand, G., *J. Canc. Res. Clin. Oncol.* 1986, *112*, 149 – 153.

[17] Park, M., Gu, F., Loeppky, R. N., *Tetrahedron Lett.* 1998, *39*, 1287 – 1290.

[18] Loeppky, R. N., Gu, F., Goelzer, P., Shi, J., *Book of Abstracts, 214th ACS National Meeting, Las Vegas, NV, September 7 – 11, 1997.*

[19] Conrad, J., Mueller, N., Eisenbrand, G., *Chem. Biol. Interact.* 1986, *60*, 57 – 65.

[20] Eisenbrand, G., Mueller, N., Denkel, E., Sterzel, W., *J. Canc. Res. Clin. Oncol.* 1986, *112*, 196 – 204.

[21] Mueller, N., Conrad, J., Eisenbrand, G., *IARC Sci. Publ.* 1987, *84*, 194 – 196.

[22] Preussmann, R., Habs, M., Habs, H., Schmaehl, D., *Canc. Res.* 1982, *42*, 5167 – 5171.

[23] Hilfrich, I., Schmeltz, I., Hoffmann, D., *Canc. Lett.* 1978, *4*, 55 – 60.

[24] Eisenbrand, G., Denkel, E., Pool, B. L., *J. Canc. Res. Clin. Onc.* 1984, *108*, 76 – 80.

[25] Scherer, G., Ludeke, B., Kleihues, P., Loeppky, R. N., Eisenbrand, G., *IARC Sci. Publ.* 1991, *105*, 339 – 342.

[26] Sterzel, W., Eisenbrand, G., *J. Canc. Res. Clin. Onc.* 1986, *111*, 20 – 24.

[27] Janzowski, C., Scharner, A., Eisenbrand, G., *Eur. J. Canc. Prev.* 1996, *5*, 154.

[28] Dittberner, U., Eisenbrand, G., Zankl, H., *J. Canc. Res. Clin. Onc.* 1988, *114*, 575 – 578.

[29] Henn, I., Eisenbrand, G., Zankl, H., *J. Canc. Res. Clin. Onc.* 1989, *115*, 445 – 448.

[30] McKelvey-Martin, V. J., Green, M. H. L., Schmezer, P., Pool-Zobel, B. L. *et al.*, *Mutat. Res.* 1993, *288*, 47 – 63.

[31] Chuang, C.-H., Hu, M.-L., *Mutat. Res*. 2004, *564*, 75 – 82.

[32] Faust, F., Kassie, F., Knasmuller, S., Boedecker, R. H. *et al.*, *Mutat. Res.* 2004, *566*, 209 – 229.

[33] Boiteux, S., O'Connor, R. T., Lederer, F., Gouyette, A., Laval, J., *J. Biol. Chem.* 1990, *265*, 3916 – 3122.

[34] Payne, G. P., Williams, P. H., *J. Org. Chem.* 1961, *26*, 651 – 659.

[35] Singh, N. P., McCoy, M. T., Tice, R. R., Schneider, E. L., *Exp. Cell Res.* 1988, *175*, 184 – 191.

[36] Collins, A. R., in: Lunec, J., Griffiths, H. R. (Eds.), *Measuring in vivo Oxidative Damage: A Practical Approach*, John Wiley and Sons, New York 2000, 83 – 94.

[37] Collins, A. R., *Molec. Biotech.* 2004, *26*, 249 – 261.

[38] Bradley, M. O., Bhuyan, B., Francis, M. C., Langenbach, R. *et al.*, *Mutat. Res.* 1981, *87*, 81 – 142.

[39] Glatt, H.-R., in: Fahrig, R. (Ed.), *Mutationsforschung und genetische Toxikologie*, Wissenschaftliche Buchgesellschaft, Darmstadt 1993, pp. 243 – 262.

[40] Janzowski, C., Glaab, V., Samimi, E., Schlatter, J., Eisenbrand, G., *Food Chem. Toxicol.* 2000, *38*, 801 – 809.

[41] Gates, K. S., Nooner, T., Dutta, S., *Chem. Res. Toxicol.* 2004, *17*, 840 – 856.

[42] Wood, A. W., Chang, R. L., Levin, W., Yagi, H. *et al.*, *Biochem. Biophys. Res. Commun.* 1977, *77*, 1389 – 1396.

[43] Cheng, S. C., Hilton, B. D., Roman, J. M., Dipple, A., *Chem. Res. Toxicol.* 1989, *2*, 334 – 340.

[44] Sage, E., Haseltine, W. A., *J. Biol. Chem.* 1984, *259*, 11098 – 11102.

[45] Speit, G., Hanelt, S., Helbig, R., Seidel, A., Hartmann, A., *Toxicol. Lett.* 1996, *88*, 91 – 98.

