Food and Chemical Toxicology 46 (2008) 2344–2348

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Nitropolycyclic aromatic hydrocarbons are inducers of mitotic homologous recombination in the wing-spot test of *Drosophila melanogaster*

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ARTICLE INFO

Article history:
Received 13 September 2007
Accepted 12 March 2008

Keywords:
SMART
Genotoxicity
NPAHs
Drosophila melanogaster
Mitotic recombination

ABSTRACT

In this study, the widespread environmental pollutants 1-nitronaphthalene (1NN), 1,5-dinitronaphthalene (1,5DNN), 2-nitrofluorene (2NF) and 9-nitroanthracene (9NA), were investigated for genotoxicity in the wing somatic mutation and recombination test (SMART) of *Drosophila* – using the high bioactivation (HB) cross. Our in vivo experiments demonstrated that all compounds assessed induced genetic toxicity, causing increased incidence of homologous somatic recombination. 2NF, 9NA and 1NN mutant clone induction is almost exclusively related to somatic recombination, although 1,5DNN-clone induction depends on both mutagenic and recombinagenic events. 1NN has the highest recombinagenic activity (~100%), followed by 2NF (~77%), 9NA (~75%) and 1,5DNN (33%). 1NN is the compound with the strongest genotoxicity, with 9NA being ~40 times less potent than the former and 2NF and 1,5DNN ~333 times less potent than 1NN. The evidence indicating that the major effect observed in this study is an increased frequency of mitotic recombination emphasizes another hazard that could be associated to NPAHs – the increment in homologous recombination (HR).

1. Introduction

Nitropolycyclic aromatic hydrocarbons (NPAHs) are widespread environmental pollutants present in both primary combustion emissions and air particulate matter. They can be formed in combustion processes, as well as in the reactions of polycyclic aromatic hydrocarbons (PAHs) with dinitrogen pentoxide, hidroxyl-, and nitrate-radicals, which are common in polluted atmospheres (Fan et al., 1996). Many of these compounds may represent a health hazard to humans, due to their widespread environmental presence and/or genotoxic activity (IARC, 1989). Numerous nitroarenes have been found to induce gene mutation and are associated with high incidence of tumors (Öztürk and Durusoy, 1999). In addition, the mutagenicity ascribed to nitroaromatic compounds seems to result from an electrophilic attack mainly at the C8-position of deoxyguanosine (Haack et al., 2001).

Much of the literature concerning air pollution comes from studies in North American and European cities (Villalobos-Pietrini et al., 2000). More recently, the effects of seasonal weather on genotoxicity, cytokinetic, cytotoxicity and organochemical content of extracts from airborne particulates collected in Mexico City were described. Twelve of the most common NPAHs present in several polluted cities were analyzed, but only eight were found in Mexico City. From these, four compounds were detected in large concentrations – 1-nitronaphthalene (1NN); 1,5-dinitronaphthalene (1,5DNN); 2-nitrofluorene (2NF) and 9-nitroanthracene (9NA) (Calderón-Segura et al., 2004). 2NF is a widely studied contaminant, being considered a model compound in studies of toxicant metabolism, carcinogenesis and mutagenesis (Heflich and Neft, 1994; Hoffmann et al., 2001). 9NA was the contaminant responsible for all tumor induction events observed in a long-term study conducted by the National Toxicology Program (Butterworth et al., 2001). 1NN metabolites were associated with tumor induction in experimental animals (El-Bayoumy and Hecht, 1982). 1,5DNN, the nitro compound least investigated, has shown weak mutagenic response in the *Salmonella typhimurium* gene mutation assay (Tokiwa et al., 1985).

Although the mutagenic and carcinogenic properties related to some nitro compounds are well documented in the literature, little is known about the recombinational potency of NPAHs. Homologous recombination (HR) can be a major mechanism in the loss of heterozygosity (LOH) required for the second step in the two-step model or for a later event in a multi-step model of

Abbreviations: 1,5DNN, 1,5-dinitronaphthalene; HR, homologous recombination; LOH, loss of heterozygosity; 9NA, 9-nitroanthracene; 2NF, 2-nitrofluorene; 1NN, 1-nitronaphthalene; NPAHs, nitropolycyclic aromatic hydrocarbons; PAHs, polycyclic aromatic hydrocarbons; SCEs, sister chromatid exchanges.

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doi:10.1016/j.fct.2008.03.014

carcinogenesis. The elevated frequencies of HR and genome rearrangements observed in cells from human patients suffering from cancer-prone diseases and the report that HR can act as an alternative mechanism of telomere maintenance pointed out that homologous mitotic recombination is one of the most important processes required for carcinogenesis. (Bishop and Schiestl, 2001, 2003).

The somatic mutation and recombination test (SMART) is based on the loss of heterozygosity (LOH) induction, which may occur through various mechanisms, such as point mutation and certain types of chromosome mutations, as well as mitotic recombination. This versatile short-term *in vivo* assay simultaneously detects mutational and mitotic recombination events, and is able to quantify the recombinagenic activity of a compound in a genotoxicity screening. To obtain more detailed knowledge concerning the genotoxic profile of four NPAHs – 1-nitronaphthalene (1NN); 1,5-dinitronaphthalene (1,5DNN); 2-nitrofluorene (2NF) and 9-nitroanthracene (9NA), we employed the high bioactivation (HB) version of the wing SMART test in *Drosophila melanogaster* (Andrade et al., 2004). The mutational and recombinational potential was quantified as well as the total genotoxicity as a function of exposure concentration was determined for all compounds – with special emphasis to their recombinagenic action in two intervals of the chromosome 3 of *D. melanogaster*.

2. Materials and methods

2.1. Chemicals

In the present study, 1-nitronaphthalene (CAS no. 86-57-7, purity 99%); 1,5-dinitronaphthalene (CAS no. 605-71-0, purity 97%); 2-nitrofluorene (CAS no. 607-57-8, purity 98%); 9-nitroanthracene (CAS no. 602-60-8, purity 90%) were purchased from Sigma–Aldrich (São Paulo, Brazil). Chemical structures of the compounds are presented in Fig. 1. All tested compounds were dissolved in 5% ethanol and 5% Tween-80 before use.

2.2. Wing somatic mutation and recombination test (SMART)

2.2.1. Strains

a. *mwh*: The marker *multiple wing hairs* (*mwh*, 3–0.3), which is a completely recessive, homozygous viable mutation, is kept in a homozygous *mwh* strain.

b. High bioactivation (HB) line: *ORR/ORR; flr^3/ In(3LR)TM3, ri p^p sep l(3)89Aa bx^{34e} e Bd^S*. The *ORR* strain has chromosomes 1 and 2 from a DDT-resistant Oregon R(R) line, which are responsible for a high constitutive level of cytochrome P450. In particular, the CYP6A2 level is increased (Saner et al., 1996), primarily as a result of mutation of the cytochrome P450 regulatory gene *Rst(2)DDT*. Information on these crosses is available in Graf and van Schaik (1992). More details about the genetic markers are given in Lindsley and Zimm (1992).

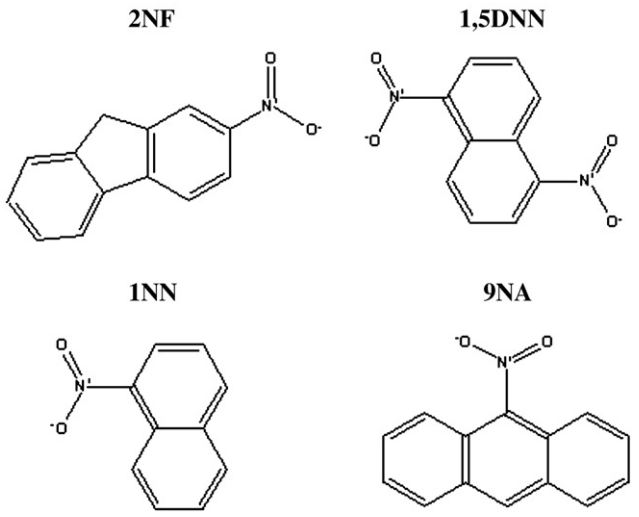

Fig. 1. Chemical structures of 2-nitrofluorene (2NF), 1,5-dinitronaphthalene (1,5DNN), 1-nitronaphthalene (1NN) and 9-nitroanthracene(9NA).

2.2.2. Culturing and treatment of tester strains

The genetic toxicity of the four compounds tested was assessed using the HB cross: *ORR/ORR; flr^3/ TM3, Bd^S* females to *mwh/mwh* males. Flies were allowed to lay eggs for 8 h in culture vials, containing a solid agar base (3% w/v) completely covered with a layer of live fermenting baker's yeast supplemented with sucrose. Approximately 72 h after the end of the egg-laying stage, larvae were collected and distributed in plastic vials containing 1.5 g of Drosophila Instant Medium (Carolina Biological Supply; Burlington, NC, USA) re-hydrated with 5 ml of the test solutions at different concentrations. Two experiments were performed with each compound and its concurrent negative controls. The larvae treated remained in the vials upon emergence of the surviving adult flies.

2.2.3. Scoring of wings

The induction of LOH in the marker-heterozygous flies produces two mutant clones: (i) single spots, either *mwh* or *flr^3*, resulting from point or chromosome mutations as well as mitotic recombination, and (ii) twin spots, consisting of both *mwh* and *flr^3* subclones, which are originated exclusively from mitotic recombination. In the balancer-heterozygous genotype, *mwh* spots reflect predominantly somatic point mutation and chromosome mutation, since mitotic recombination involving the balancer chromosome and its structurally normal homologue is a lethal event (Andrade et al., 2004).

2.2.4. Statistical analysis

To evaluate the statistical significance of the results obtained, we followed a multiple decision procedure of Frei and Würgler (1988), which makes four different diagnoses: positive, weakly positive, negative or inconclusive. The frequency of each type of mutant clone per fly of a treated series was compared pair-wise (i.e., negative control vs. tested compounds) using the conditional binomial test of Kastembaum and Bowman (1970). The recombinagenic action of the drugs was calculated comparing the standard frequency of clones/10^5 cells obtained from *mwh/flr^3* and *mwh/TM3* genotypes (Frei and Würgler, 1996). For an unbiased comparison of this frequency just *mwh* clones in *mwh* single spots and in twin spots were used (Frei et al., 1992).

3. Results

The overall genetic toxicity results obtained by means of the analysis of both marker-trans-heterozygous (*mwh/flr^3*) and balancer-heterozygous (*mwh/TM3*) genotypes from the nitroaromatic compounds are presented in Table 1. For each concentration, the table shows the total number of flies analyzed, the frequency of the different mutant clones, as well as the total spots scored, which represent the final genotoxicity of the compound tested. In the case of positive statistical diagnosis, the (*mwh/TM3*) flies were also scored, which afforded to quantify the contribution of mutagenic and recombinagenic events to the final genotoxicity observed. The negative control frequencies of total spots per fly ranged from 0.97 to 1.00 for the *mwh/flr^3* genotype and from 0.87 to 0.90 for the *mwh/TM3* genotype, being in accordance with the usual range previously reported in the literature (Würgler et al., 1985; Dihl et al., 2008). Prior to the genetic toxicity assessment, the organic compounds were submitted to a dose range test (data not shown), which demonstrated that 1NN presented the highest toxicity, followed by 9NA, 1,5DNN and 2NF, respectively in larvae fed for 48 h on each chemical. Non-toxic concentrations were used to perform the genotoxic evaluation of these drugs. It turned out that severity of toxic effect was similar for 1,5DNN and 2NF so that the four doses ranges presented in Table 1 for 9NA and 1NN do not overlap.

3.1. Genetic toxicity

As seen in Table 1, all four chemicals showed positive effect in the trans-heterozygous larvae, producing statistically significant increases in total spot frequencies, which means that they are clearly active in this test system. The data obtained also indicated that their genotoxic effects are related to increases in the small single spot frequencies in almost all concentrations assessed,