

Arylamines Suppress Their Own Activation and That of Nitroarenes in V79 Chinese Hamster Cells by Competing for Acetyltransferases

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The effect of 2-aminofluorene (2-AF) on the toxicity of 2-aminoanthracene (2-AA) and 1,6-dinitropyrene (1,6-DNP) was studied in *N*-acetyltransferase-proficient V79-NHr1A2 cells genetically engineered for the expression of cytochrome P4501A2, and in wild-type V79-NH cells. 2-AA inhibited the growth of V79-NHr1A2 cells and induced the formation of micronuclei at concentrations of 0.1 to 1.0 μ M, but was virtually without toxic effects at a concentration of 10 μ M. Addition of 2-AF protected against the cytotoxic and genotoxic effects elicited by low concentrations of 2-AA. Half-maximum protection was observed at 0.2 to 0.5 μ M 2-AF. The arylamine also prevented the cytotoxicity caused by 1,6-DNP in V79-NH cells and completely suppressed the formation of 1-acetyl-amino-6-nitropyrene from 1,6-DNP in these cells. The results indicate that arylamines and related *N*-hydroxyarylamines are substrates for the same acetyltransferase in V79-NH cells. In consequence, arylamines are capable of suppressing the activation of their proximate cytotoxic and genotoxic products in these cells and, presumably, *in vivo*. — Environ Health Perspect 102(Suppl 6):95–97(1994)

Key words: acetyltransferase, 2-aminoanthracene, 2-aminofluorene, arylamines, cytotoxicity, genotoxicity, 1,6-dinitropyrene, micronuclei, V79-NH cells, V79-NHr1A2 cells

Introduction

The activation of arylamines involves *N*-hydroxylation by microsomal cytochrome P450 (P450), notably P4501A2, and *O*-acetylation of the *N*-hydroxylated products by cytosolic transferases (1,2). Several observations suggest that the *N*-acetylation of arylamines such as 2-aminofluorene (2-AF), the *O*-acetylation of their *N*-hydroxylated products, and the *N*,*O*-transacetylation of their hydroxamic acids are mediated by the same enzyme (3–5). The possible effects of this overlap in substrate specificities on the toxicokinetics and biologic effects of arylamines are not yet clear.

The present study was aimed at investigating whether, and to what degree, arylamines may interfere with their secondary activation to cytotoxic and genotoxic products. To this end, we studied the effects of 2-AF on the toxicity of 2-aminoanthracene (2-AA) and 1,6-dinitropyrene (1,6-DNP) in the newly established Chinese hamster cell line V79-NHr1A2, a V79-NH-derived

construct expressing P4501A2, and in the parental V79-NH cells.

Materials and Methods

Cells

The V79 cell strain used, V79-NH, lacks significant cytochrome P450 activities (6) but contains the monomorphic and polymorphic *N*-acetyltransferase (7). V79-NHr1A2 cells were derived from V79-NH cells by genetic engineering for stable expression of rat cytochrome P4501A2 as has been described for another V79 cell strain, V79-MZ (8). Cells were cultured as described by Kiefer and Wiebel (6).

Experimental Schedule

Cells were seeded on microtiter plates at a density of 500 cells/well, or on 60 mm plates at a density of 5×10^4 cells, for the determination of cytotoxicity or genotoxicity, respectively. After 24 hr of growth they were exposed to the test chemicals for 72 hr.

Determination of Cytotoxic and Genotoxic Effects

Cytotoxicity was determined by measuring neutral red uptake according to Borenfreund and Puerner (9) or DNA content as described by Goettlicher et al. (10). Genotoxicity was assayed by determining

the frequency of micronuclei as described by Roscher and Wiebel (11).

Metabolism of 1,6-Dinitropyrene

Cells grown to a density of $5\text{--}10 \times 10^6/100$ mm plate were exposed to 1 μ M ^{14}C -1,6-DNP, specific activity 56.5 mCi/mmol, for 8 or 24 hr. Aliquots of medium/cell suspensions were extracted with two volumes of chloroform/methanol (1:1). Organic soluble metabolites were separated on reverse-phase thin-layer plates at 4°C using methanol as mobile phase. They were quantitated by autoradiography (Digital Autoradiograph, Berthold, Wildbad, Germany). Aqueous soluble material amounted to approximately 10% of the total radioactivity in the medium/cell suspensions.

Results and Discussion

Cytotoxicity and Genotoxicity of 2-Aminoanthracene in V79-NHr1A2 Cells: Concentration Dependency and Protective Effect of 2-Aminofluorene

2-AA inhibited the growth of V79-NHr1A2 cells and induced the formation of micronuclei in a concentration-dependent manner causing maximum effects at 0.3 μ M (Figure 1, left panels). Higher concentrations of 2-AA reversed the toxic effects. Cellular growth and frequency of

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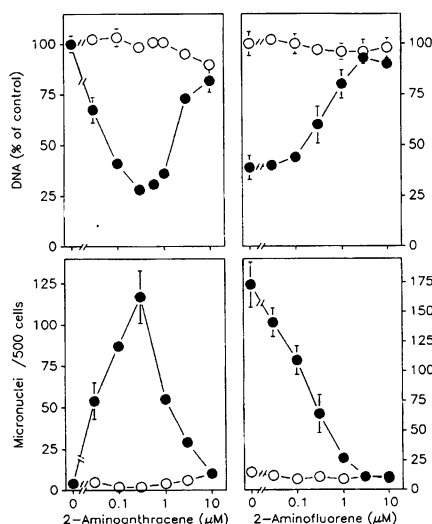


Figure 1. Cytotoxicity and genotoxicity of 2-AA in V79-NHr1A2 cells: protective effect of 2-AF. Panels to the left: (●—●) 2-AA alone, (○—○) 2-AA and 10 μM 2-AF; panels to the right: (○—○) 2-AF alone, (●—●) 2-AF and 0.6 μM 2-AA (upper panel) or 0.3 μM 2-AA (lower panel). Test substances were added in DMSO. Maximum final solvent concentrations were 0.2%. Other conditions as described in "Materials and Methods."

micronuclei were restored almost to normal at 10 μM 2-AA.

Addition of 10 μM 2-AF to V79-NHr1A2 cultures exposed to low concentrations of 2-AA completely protected against the toxic effects of the latter (Figure 1). The concentration of 0.2 to 0.5 μM 2-AF resulted in half-maximum protection against the toxicity of 0.3 μM 2-AA (Figure 1, right panels). 2-AF alone affected neither the growth of V79-NHr1A2 cells nor the frequency of micronuclei in these cells (Figure 1, right panels).

At present, we cannot exclude the possibility that 2-AF exerts its protective effect by inhibiting the *N*-hydroxylation of 2-AA. However, the unusual concentration dependency observed for the toxicity of 2-AA argues against this possibility. 2-AF, and related arylamines, are more likely to act by suppressing the *O*-acetylation of their primary hydroxylation products, i.e., by competing for a common acetyltransferase. This is supported by findings

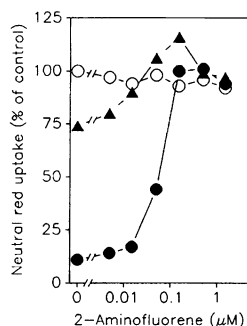


Figure 2. Protection of V79-NH cells against 1,6-DNP-induced cytotoxicity by 2-AF. (▲—▲) 0.5 μM 1,6-DNP and 2-AF, (●—●) 5 μM 1,6-DNP and 2-AF, (○—○) 2-AF alone. 1,6-DNP was added in DMSO. Maximum final solvent concentrations were 0.3%. Other conditions as described in "Materials and Methods."

described below which show that 2-AF also protects against the toxicity of 1,6-DNP.

Toxicity and Metabolism of 1,6-Dinitropyrene in V79-NH Cells: Effect of 2-Aminofluorene

Since nitroarenes such as 1,6-DNP are activated via reduction to *N*-hydroxyarylamines and subsequent *O*-acetylation (12), their toxicity may also be suppressed by arylamines. Previous investigations (11) have shown that V79-NH cells are highly sensitive to the toxic effects of 1,6-DNP. This sensitivity was found to be closely associated with the capability of these cells for *N*-acetylating 1-amino-6-nitropyrene (7).

As shown in Figure 2, the growth inhibitory effect of 0.5 and 5.0 μM 1,6-DNP was reduced by 50% in the presence of as little as 0.03 to 0.05 μM 2-AF. In the presence of 0.1 μM 2-AF, the cells were fully protected even against 5.0 μM 1,6-DNP. Other arylamines such as 2-AA also prevented the toxicity of 1,6-DNP in V79-NH cells (data not shown).

If the hypothesis that arylamines exert their protective effect by competing for an acetyltransferase were correct, 2-AF should counteract the acetylation of 1,6-DNP reduction products but not block the process of 1,6-DNP reduction itself. In fact, this was the case. As shown in Figure 3 (left panel), V79-NH cells exposed to 1,6-DNP for 8 hr formed sizable amounts

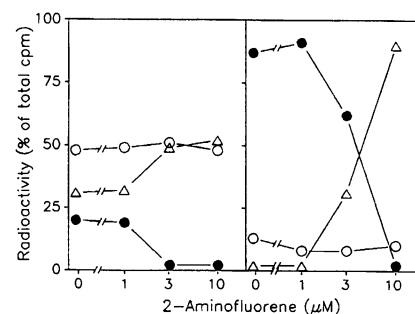


Figure 3. Metabolism of 1,6-DNP in V79-NH cells: effect of 2-AF. Cells were exposed to 1 μM ¹⁴C-1,6-DNP in the presence of 1, 3, and 10 μM 2-AF for 8 hr (left panel) or 24 hr (right panel). (●—●) 1-Acetyl-6-nitropyrene, (△—△) 1-amino-6-nitropyrene, (○—○) 1,6-DNP. The ordinate presents radioactivity as percent of total radioactive, organic soluble material. The rate of 1,6-DNP metabolism was 19 pmole/10⁶ cells × hr during the first 8 hr of exposure. Other conditions as described in "Materials and Methods."

of 1-amino-6-nitropyrene and 1-acetyl-6-nitropyrene. After 24 hr of exposure, virtually all of the 1,6-DNP was converted to 1-acetyl-6-nitropyrene (right panel). 2-AF, at 3 to 10 μM concentrations, strongly inhibited the formation of the acetylated product and, correspondingly, increased the appearance of 1-amino-6-nitropyrene. Thus the total metabolism of 1,6-DNP remained unaffected by the arylamine.

Conclusion

The results suggest that arylamines are capable of competing with their *N*-hydroxylated products, and the reduction products of nitroarenes, for the same acetyltransferase(s), thereby suppressing the formation of the ultimate genotoxic products. It is possible that the phenomenon is specific to cultured cells, in particular Chinese hamster cells such as the V79-NH cells and the P4501A2-containing cell constructs used in this study. However, it is equally likely that arylamines also interfere with their own activation *in vivo* resulting in a highly complex dose dependency in experimental animals and man.

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