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The genotoxic air pollutant 3-nitrobenzanthrone and its reactive metabolite *N*-hydroxy-3-aminobenzanthrone lack initiating and complete carcinogenic activity in NMRI mouse skin

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ABSTRACT

3-Nitrobenzanthrone (3-NBA), a genotoxic mutagen found in diesel exhaust and ambient air pollution and its active metabolite *N*-hydroxy-3-aminobenzanthrone (*N*-OH-3-ABA) were tested for initiating and complete carcinogenic activity in the NMRI mouse skin carcinogenesis model. Both compounds were found to be inactive as either tumour initiators or complete carcinogens in mouse skin over a dose range of 25–400 nmol. Topical application of 3-NBA and *N*-OH-3-ABA produced DNA adduct patterns in epidermis, detected by ^{32}P-postlabelling, similar to those found previously in other organs of rats and mice. 24 h after a single treatment of 100 nmol DNA adduct levels produced by 3-NBA (18 ± 4 adducts/10^8 nucleotides) were 6 times lower than those by 7,12-dimethylbenz[*a*]anthracene (DMBA; 114 ± 37 adducts/10^8 nucleotides). In contrast, identical treatment with *N*-OH-3-ABA resulted in adduct levels in the same range as with DMBA (136 ± 25 adducts/10^8 nucleotides), indicating that initial DNA adduct levels do not parallel tumour initiating activity. When compounds were tested for tumour initiating activity by a single treatment followed by twice-weekly applications of TPA, DNA adducts formed by DMBA, but not by 3-NBA or *N*-OH-3-ABA, were still detectable 40 weeks after treatment. When tested for activity as complete carcinogens by twice-weekly topical application, 3-NBA and *N*-OH-3-ABA produced identical DNA adduct profiles in mouse skin, with adducts still detectable after 40 weeks. Only 3-NBA produced detectable adducts in other organs.

1. Introduction

A large body of evidence supports the concept that carcinogenesis is a multistage process and its stages have been defined experimentally as initiation, promotion and progression [1]. Thus, models mimicking this process, especially the multistage mouse skin carcinogenesis model, can serve as powerful methods for the study of cancer induction and chemoprevention [2–4]. Initiation involves mutation of cellular DNA resulting in the activation of proto-oncogenes (*e.g. ras*) and inactivation of tumour suppressor genes [5,6]. Initiation is thought to be irreversible consisting of a single gene mutation caused by environmental genotoxic agents [7]. Clonal expansion of these initiated cells due to promoting stimuli, for example by application of potent phorbol ester-type skin tumour promoters, is the driving force for development of skin papillomas that may develop into squamous cell carcinoma [8]. Currently, the two-stage mouse skin carcinogen-

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esis protocol consists of an initiation phase by the administration of a single low dose of a carcinogen such as 7,12-dimethylbenz[*a*]anthracene (DMBA) and a promotion phase involving repeated application of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) [9]. This protocol provides a rapid response with relative ease of quantification of various parameters of tumourigenic response, including tumour incidence, latency, multiplicity and malignancy [2]. Moreover, as shown for DMBA [10] this model system offers a rapid approach to investigate distinct molecular mechanisms of tumour development including mutation analysis in critical target genes (*e.g. ras*) for carcinogenesis. The two-stage mouse skin carcinogenesis model has been used not only to examine the carcinogenic potential of single genotoxic agents [11,12] but also to evaluate the cancer risk of complex environmental mixtures such as cigarette smoke condensate, coal tar and diesel exhaust extracts [4,13–16].

Numerous epidemiological studies have found increased mortality and morbidity from respiratory and cardiovascular diseases associated with exposures to ambient air pollution [17,18]. A complex variety of genotoxins has been detected in urban air pollution [19] and high exposures are associated with an increased risk of cancer. Polycyclic aromatic hydrocarbons (PAHs) and nitropolycyclic aromatic hydrocarbons (nitro-PAHs) are present in particulate matter from direct atmospheric emission, such as diesel and gasoline exhaust [19–21]. Nitro-PAHs often have greater mutagenic and carcinogenic properties compared to their parent PAHs, and their persistence in the environment has led to considerable interest in assessing their potential risk to humans [22–24].

The aromatic nitroketone 3-nitrobenzanthrone (3-NBA; 3-nitro-7H-benz[*de*]anthracen-7-one; Fig. 1), identified in diesel exhaust and ambient air pollution [25,26], is one of the most potently mutagenic compounds ever detected in the Salmonella reverse mutation assay, and it is a suspected human carcinogen [25,27]. Its isomer 2-nitrobenzanthrone has also been detected in urban air particulate matter but has a much lower genotoxic potential [28–30]. 3-NBA forms DNA adducts *in vitro* and in rodents *in vivo* after metabolic activation through reduction of the nitro group, primarily catalysed by cytosolic nitroreductases such as NAD(P)H:quinone oxidoreductase (NQO1) (Fig. 1) [31–35]. The uptake of 3-NBA in humans has been demonstrated by the detection of 3-aminobenzanthrone (3-ABA; Fig. 1), its main metabolite, in the urine of workers occupationally exposed to diesel emissions [36]. The genotoxicity of 3-ABA has been demonstrated in several short-term assays [37,38]. 3-ABA is predominantly activated by cytochrome P450 (CYP) enzymes, namely CYP1A1 and CYP1A2 [39,40]. Both 3-NBA and 3-ABA can be further activated by *N*-acetyltransferases (NATs) and sulfotransferases (SULTs), *N*-hydroxy-3-aminobenzanthrone (*N*-OH-3-ABA; Fig. 1) being the reactive intermediate [33,41,42]. The predominant DNA adducts detected by ^{32}P-postlabelling *in vivo* in rodents after treatment with either 3-NBA or 3-ABA are 2-(2′-deoxyguanosin-N^2-yl)-3-aminobenzanthrone (dG-N^2-ABA) and *N*-(2′-deoxyguanosin-8-yl)-3-aminobenzanthrone (dG-C8-*N*-ABA) [43,44], and these are most probably responsible for the GC → TA transversion mutations induced by 3-NBA exposure *in vitro* and *in vivo* [45,46]. These DNA adducts not only represent premutagenic lesions in DNA, but they may also be of primary importance for tumour development in target tissues [27,47,48]. The mouse skin carcinogenesis model may provide a powerful tool to examine the molecular mechanism(s) involved in tumour initiation by 3-NBA (*e.g.* oncogene activation) and to correlate mutations in critical target genes (*e.g. ras* protooncogene) found in the tumours with promutagenic DNA adducts formed by this rodent carcinogen.

In the present study we have explored and assessed 3-NBA and its reactive metabolite *N*-OH-3-ABA for carcinogenicity and tumour initiating activity in mouse skin. In addition, DNA adduct formation was investigated using

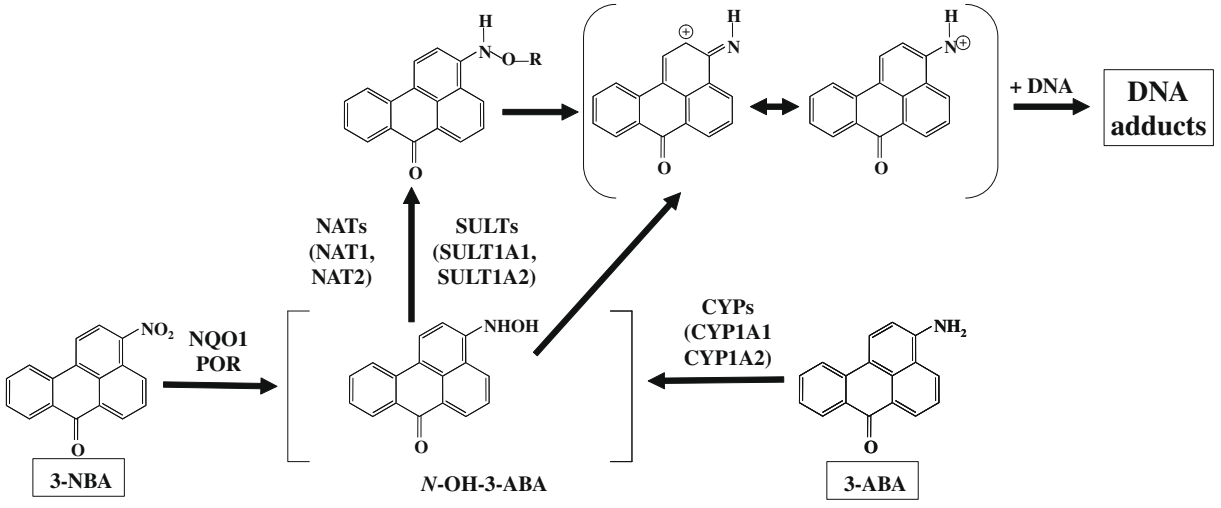

Fig. 1. Proposed pathways of metabolic activation and DNA adduct formation of 3-NBA and *N*-OH-3-ABA. See text for details. POR, cytochrome P450 oxidoreductase. R = –C(O)CH_3 or –SO_3H.