Mutagenesis vol.5 no.4 pp.375–380, 1990

Involvement of different pathways in the genotoxicity of nitropropanes in cultured mammalian cells

Eike Roscher, Kyriakoula Ziegler-Skylakakis and Ulrich Andrae[1]

GSF-Institut für Toxikologie, D-8042 Neuherberg/München, FRG

[1]To whom correspondence should be addressed

The metabolic pathways leading to genotoxicity of nitropropanes in mammalian cells were investigated by measuring the effects of 2-nitropropane (2-NP) and 1-nitropropane (1-NP) on various cell lines characterized for their expression of cytochrome P450-dependent mono-oxygenases. Cells used were the rat hepatoma cell lines 2sFou, H4IIEC3/G⁻ and C2Rev7, which express various forms of cytochrome P450-dependent mono-oxygenases, and V79 Chinese hamster cells which lack these enzyme activities. Induction of DNA repair synthesis, micronuclei and, where assessable, mutations to 6-thioguanine (TG) resistance served as indicators of genotoxic effects. 2-NP elicited a positive response at all endpoints measured in the hepatoma lines after pretreatment of the cells with dexamethasone, an inducer of various liver-specific cytochrome P450 forms. Genotoxicity was much weaker or not detectable in cells not pretreated with the inducer. 1-NP was not genotoxic in the hepatoma cells irrespective of whether the cells were pretreated or not. Neither isomer elicited DNA repair synthesis in V79 cells, but both isomers caused mutations to TG resistance, and 1-NP increased the number of micronucleated and multinucleated cells. The findings show that there are different pathways in mammalian cells by which nitropropanes can be converted to genotoxic products. Presumably the induction of liver tumours by 2-NP is linked to the metabolic pathway which is characterized by the formation of genotoxic metabolites from 2-NP but not 1-NP. This pathway appears to depend on the presence of liver-specific, dexamethasone-inducible, cytochrome P450 forms. The relevance of the genotoxic effects of the nitropropanes observed in V79 cells for the situation *in vivo* is open to question.

Introduction

1-Nitropropane (1-NP) and 2-nitropropane (2-NP) are used as industrial solvents and intermediate products for chemical syntheses. 2-NP induces liver tumours in rats following exposure by inhalation (Lewis *et al.*, 1979; Griffin *et al.*, 1980) or gavage (Fiala *et al.*, 1987), whereas 1-NP is not carcinogenic (Hadidian *et al.*, 1968; Griffin *et al.*, 1982; Fiala *et al.*, 1987). The hepatocarcinogenicity of 2-NP is most likely the consequence of DNA damage induced in the livers of the animals since 2-NP elicits DNA repair synthesis in rat liver *in vivo* as well as in cultured rat hepatocytes (Andrae *et al.*, 1988). However, 2-NP does not induce DNA repair in various cell lines of extrahepatic origin, suggesting that liver-specific metabolism is required for the formation of DNA-damaging metabolites from 2-NP (Andrae *et al.*, 1988). After treatment with 1-NP no repair synthesis was detected in liver cells *in vivo* or *in vitro*, or in any of the cell lines previously examined (Andrae *et al.*, 1988).

Although various enzymatic and non-enzymatic routes have been identified by which 2-NP can be degraded *in vitro*, the pathway leading to the formation of genotoxic and carcinogenic products is not known. We have therefore investigated the genotoxicity of the nitropropanes in various cell lines characterized for their expression of cytochrome P450-dependent mono-oxygenases in order to assess the possible role of mono-oxygenase-dependent and independent pathways in the formation of reactive metabolites of 2-NP and 1-NP. The cells used express either both the ubiquitous form of the P450I class and liver-specific forms of the P450II class (H4IIEC3/G⁻ and 2sFou rat hepatoma cells), only the latter type of enzymes (C2Rev7 rat hepatoma cells) or no detectable cytochrome P450 at all (V79 Chinese hamster cells) (Wiebel *et al.*, 1984 a–c; Corcos and Weiss, 1988; for nomenclature of cytochrome P450, see Nebert *et al.*, 1989). Genotoxicity was estimated by measuring the induction of DNA repair synthesis and micronuclei. In the case of H4IIEC3/G⁻ and V79 cells, we also determined the capacity of the nitropropanes to increase the frequency of 6-thioguanine (TG)-resistant mutants.

Materials and methods

Materials

Chemicals and cell culture materials were obtained from the following sources: 2-NP (>99% pure) was a gift from Angus Chemie, Ibbenbüren-Uffeln; 1-NP was purchased from Merck, Darmstadt, and purified by distillation (final purity 97.4%, major impurity 2.3% 2-NP); 5-bromodeoxyuridine (BrdUrd), 5-fluorodeoxyuridine (FdUrd), dexamethasone (DEX) and TG from Sigma, München; foetal calf serum (FCS) from Gibco/BRL, Eggenstein; CsCl (analytical grade) from Paesel, Frankfurt; Cs_2SO_4 (analytical grade) and Mayer's haematoxylin solution from Merck, Darmstadt; methyl methanesulphonate from Merck-Schuchardt, Hohenbrunn; [5-^{3}H]deoxycytidine ([^{3}H]dCyd, 18Ci/mmol) and [methyl-^{3}H]thymidine ([^{3}H]dThd, 44 Ci/mmol) from Amersham, Braunschweig; bisbenzimide (H 33258) from Riedel-de Haen, Seelze; and cell culture media from Biochrom, Berlin (all FRG).

Cells

The cells used in this study were V79 Chinese hamster lung cells, and the cell lines H4IIEC3/G⁻, 2sFou and C2Rev7 derived from Reuber H35 rat hepatoma cells (Reuber, 1961; Pitot *et al.*, 1964). The hepatoma lines were established by Dr M.C.Weiss and co-workers (Institut Pasteur, Paris) and kindly provided by Dr F.J.Wiebel, GSF, Neuherberg. H4IIEC3/G⁻ and 2sFou cells express cytochrome P450 forms inducible by polycyclic aromatic hydrocarbons (P450IA1) as well as liver-specific forms (e.g. P450IIB1/2 and P450IIC6), whereas C2Rev7 cells only express forms of the latter type (Wiebel *et al.*, 1984 a–c; Corcos and Weiss, 1988; Roscher and Wiebel, 1988). The cytochrome P450 forms can be induced in a similar pattern by treating the cells with DEX or phenobarbital (Corcos and Weiss, 1988). V79 cells lack detectable cytochrome P450 activities (Wiebel *et al.*, 1984c).

H4IIEC3/G⁻ cells were maintained in minimal essential medium (MEM) with Earle's salts supplemented with 4 μg/ml hypoxanthine, 0.7 μg/ml thymidine and 10% FCS. C2Rev7 and 2sFou cells were routinely cultured in Ham's F12:NCTC 135 medium (1:1) containing 5% FCS. The medium was changed to the medium described above 1–2 weeks before treatment of the cells with test compounds. V79 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS. All media contained 100 U/ml penicillin and 100 μg/ml streptomycin. The cells were kept at 37°C in an atmosphere of 93% air and 7% CO_2.

Downloaded from http://mutage.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

Pretreatment of cells with DEX

In some experiments hepatoma cells were treated with medium containing 2 μM DEX for 24 h prior to exposure to the nitropropanes in order to induce monooxygenase activities. Stock solutions of DEX were made up with dimethylsulphoxide (DMSO). The concentration of the solvent in the medium was 0.1%.

Treatment of cells with nitropropanes

The hepatoma cells were seeded in 25 or 75 cm^2-flasks at densities of $1.5-2\times10^5$ or $5-6\times10^5$ cells/flask, respectively, and grown for 4 days before exposure to the test compounds. V79 cells were plated at densities of $0.5-1\times10^6$ cells in 75 cm^2 flasks 1 day before treatment. The nitropropanes were dissolved in medium by thorough shaking. The culture flasks were closed air-tight. Cells were exposed to the substances for 3–24 h depending on proliferation rates of cell lines and biological endpoint investigated. Subsequently, the cells were rinsed and fed with fresh medium.

DNA repair synthesis

DNA repair synthesis in the hepatoma cells was determined by means of the BrdUrd density-shift method (Pettijohn and Hanawalt, 1964) as modified for use with hepatocytes (Rossberger and Andrae, 1985). Following pre-incubation of the cells in medium containing 40 μM FdUrd and 200 μM BrdUrd for 1 h they were incubated with FdUrd, BrdUrd, the test compound and 10 μCi/ml [^{3}H]dCyd for 5 h. For the measurement of repair in V79 cells, FdUrd and BrdUrd concentrations of 2 and 10 μM, respectively, were used and radioactive labelling of the DNA was performed with 10 μCi/ml [^{3}H]dThd in the presence of 2 mM hydroxyurea (Smith and Hanawalt, 1976). Following incubation the DNA was isolated and unreplicated DNA strands were separated from replicated, density-labelled strands by equilibrium centrifugation in alkaline $CsCl-Cs_2SO_4$ gradients. Repair synthesis was determined by measuring the incorporation of radioactivity in unreplicated DNA strands and expressed as counts per minute per microgram DNA.

Micronuclei and multinucleated cells

Nuclei and micronuclei were prepared for counting 24 h after treatment as described previously (Ziegler-Skylakakis *et al.*, 1989). Treatment periods were 3 h for V79 cells and 24 h for the hepatoma cells. Frequencies of micronuclei, multinucleated cells and mitoses were determined by counting at least 3×500 cells in randomly selected fields by fluorescence microscopy. Structures which were characterized by (i) fluorescence similar to that of the nucleus, (ii) size up to 25% of the nuclear area and (iii) distinct separation from the nucleus, were regarded as micronuclei. Cells containing up to four micronuclei were considered as micronucleated. Cells with (i) more than four micronuclei, (ii) several nuclei of similar size or (iii) heavily fragmented nuclei were regarded as multinucleated. Background frequencies of micronuclei occasionally differed between individual cell batches. These variations, however, did not affect the response of the cells to the nitropropanes.

Cytotoxicity

Cytotoxicity of the test compounds in V79 and H4IIEC3/G$^-$ cells was measured by comparing the cloning efficiencies of treated and untreated cells. Immediately after exposing V79 cells for 3 h and H4IIEC3/G$^-$ cells for 24 h to the agents, 100–200 cells per dish were replated in growth medium in six 100 or 60 mm dishes. Colonies of V79 cells were fixed with methanol and stained with Mayer's haematoxylin solution after 6 days of undisturbed growth. H4IIEC3/G$^-$ cells were fed after 7 days, and colonies fixed and stained on the 14th day as described above.

Mutagenicity

Mutagenicity of 2-NP and 1-NP was estimated in V79 and H4IIEC3/G$^-$ cells by measuring the induction of clones resistant to 11 μg/ml TG as described previously (Roscher and Wiebel, 1988; Ziegler-Skylakakis *et al.*, 1989). Treatment periods with the nitropropanes were 3 h for V79 cells and 24 h for H4IIEC3/G$^-$ cells. Mutation frequencies were expressed as TG resistant clones/10^6 clonable cells. C2Rev7 and 2sFou cells are TG resistant and, therefore, do not allow the detection of mutants by means of TG selection.

Aldrin epoxidase activity

Aldrin epoxidase activity in the hepatoma cells was determined according to Wolff *et al.* (1979) with the modifications described recently (Roscher and Wiebel, 1988).

Results

Treatment of the cell lines with 2-NP and 1-NP resulted in cytotoxic and genotoxic effects which greatly differed between the test compounds as well as the various cell lines.

Morphological alterations

Exposure of the cells to 2-NP did not lead to marked alterations in cellular morphology, irrespective of the cell line tested. In contrast, 1-NP caused clearly discernible changes. Treatment of the hepatoma cells resulted in coagulation of the cytoplasm and a 'balloon'-like appearance of the cell membrane. This effect was slightly diminished in cultures pretreated with DEX. Morphology of V79 cells was altered in a different way: the cells appeared enlarged, and many of them contained two or more nuclei.

DNA repair synthesis

The induction of DNA lesions subject to excision repair was determined by measuring the effects of the nitropropanes on DNA repair synthesis in the four cell lines. 2-NP weakly induced DNA repair in C2Rev7 cells (Figure 1A) and in 2sFou cells (Figure 2A) but not in H4IIEC3/G$^-$ or V79 cells (Figures 3A and 4A). Pretreatment of the hepatoma cells with DEX strongly increased repair synthesis in the C2Rev7 and 2sFou cells (Figures 1A and 2A), and resulted in a slight increase in the H4IIEC3/G$^-$ cells (Figure 3A).

In contrast to 2-NP, 1-NP did not cause DNA repair in any of the cell lines tested (Figures 1B–4B).

Micronuclei

In order to assess the potential of 2-NP and 1-NP to cause chromosomal disorders, the frequencies of micronuclei indicating the appearance of structural or numerical chromosome aberrations were determined in the cell lines following exposure to the nitropropanes.

2-NP did not distinctly alter the frequency of micronuclei in hepatoma cell lines not pretreated with DEX, but it significantly enhanced the formation of micronuclei when the cells were

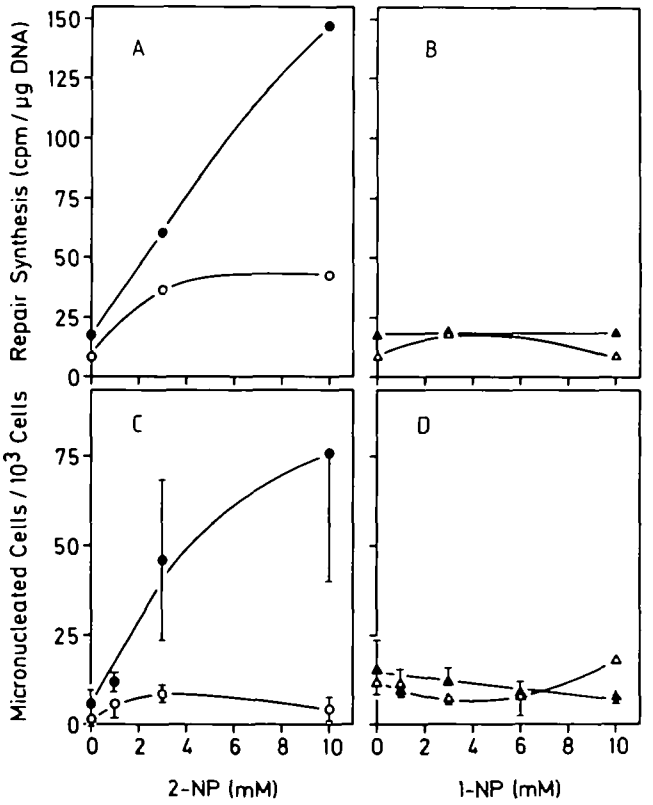

Fig. 1. DNA repair synthesis and micronuclei in C2Rev7 cells exposed to 2-NP or 1-NP. Following pre-incubation of the cells in the presence (●,▲) or absence (○,△) of 2 μM DEX for 24 h, cells were exposed to the nitropropanes for either 5 h (DNA repair, A,B) or 24 h (micronuclei, C,D). Data are from one experiment, a second experiment yielded very similar results. Data in (C) and (D) are the means ± SD of 3×500 cells. When no error bars are given, SD was below the size of the graphic symbols.

Downloaded from http://mutage.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

pretreated (Figures 1C–3C). 2-NP had no effect on V79 cells (Figure 4C).

1-NP did not influence the frequency of micronuclei in the hepatoma cell lines irrespective of whether the cells had been pretreated with DEX or not (Figures 1D–3D) whereas the frequency was increased in V79 cells (Figure 4D). This stimulation of micronuclei formation occurred in parallel with an increase in the frequency of multinucleated cells (Figure 4D) which could not be observed in V79 cells after treatment with 2-NP (Figure 4C) or in the hepatoma cells with either of the nitropropanes (data not shown).

In general, the mitotic indices were not significantly altered following exposure to the nitropropanes, with the exception of V79 cells and DEX-pretreated H4IIEC3/G⁻ cells which displayed a mitotic block after treatment with 1-NP. A prolongation of the period between the end of treatment and fixation of cells restored the mitotic indices in H4IIEC3/G⁻ cells but did not influence the frequencies of micronuclei (data not shown).

Cytotoxicity and mutagenicity

2-NP was cytotoxic to H4IIEC3/G⁻ cells only after pretreatment of the cells with DEX. Under this condition cloning efficiency was reduced to ~50% at 10 mM, the highest concentration tested (Figure 5). 1-NP was similarly cytotoxic (data not shown). Both nitropropanes were only marginally cytotoxic in V79 cells over the entire concentration range tested (0.3–10 mM, Figure 6).

The capacity of 2-NP and 1-NP to induce mutations was examined in H4IIEC3/G⁻ and V79 cells by determining the effects of the compounds on the frequencies of TG resistant cells.

2-NP was not mutagenic in H4IIEC3/G⁻ cells without DEX pretreatment, but it was clearly effective in these cells when they had been pretreated with the inducer (Figure 5). 2-NP also caused mutations in V79 cells (Figure 6). Pretreatment of V79 cells with DEX did not alter mutation induction by 2-NP (data not shown).

1-NP was not mutagenic in either DEX-treated or untreated H4IIEC3/G⁻ cells (data not shown). In V79 cells, however, it clearly increased the number of TG resistant mutants (Figure 6). In these experiments, which were performed on a batch of V79 cells different from that used for the experiments on 2-NP, 3 mM 2-NP was included as an internal standard to allow a comparison of the relative mutagenicities of 1-NP and 2-NP. As shown in Figure 6, the mutagenic efficiency of the two nitropropanes was very similar.

Induction of cytochrome P450 by DEX

The effect of the DEX pretreatment on cytochrome P450-dependent mono-oxygenases of the hepatoma cells was determined by measuring aldrin epoxidation, a function of various liver specific cytochrome P450 forms (Wolff and Guengerich, 1987). Table I shows the results obtained. After treatment of the cells with DEX, aldrin epoxidase activity was increased 3-fold in C2Rev7 cells and 2-fold in 2sFou cells. No induction was observed in H4IIEC3/G⁻ cells.

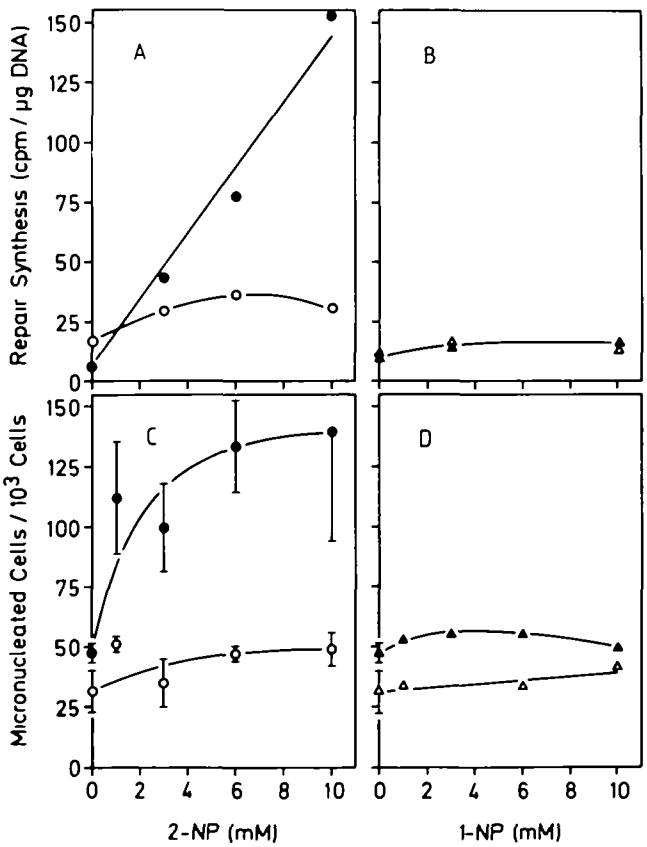

Fig. 2. DNA repair synthesis and micronuclei in 2sFou cells exposed to 2-NP or 1-NP. Following pre-incubation of the cells in the presence (●,▲) or absence (○,△) of 2 µM DEX for 24 h, cells were exposed to the nitropropanes for either 5 h (DNA repair, A,B) or 24 h (micronuclei, C,D). Data are from one experiment, a second experiment yielded very similar results. Data in (C) and (D) are the means ± SD of 3×500 cells. When no error bars are given, SD was below the size of the graphic symbols.

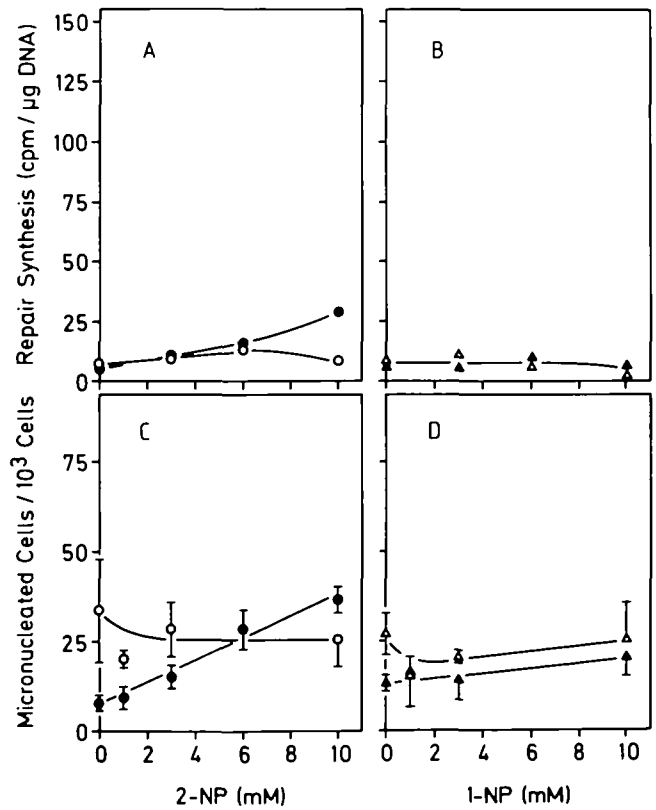

Fig. 3. DNA repair synthesis and micronuclei in H4IIEC3/G⁻ cells exposed to 2-NP or 1-NP. Following pre-incubation of the cells in the presence (●,▲) or absence (○,△) of 2 µM DEX for 24 h, cells were exposed to the nitropropanes for either 5 h (DNA repair, A,B) or 24 h (micronuclei, C,D). Data are from one experiment, a second experiment yielded very similar results. Data in (C) and (D) are the means ± SD of 3×500 cells. When no error bars are given, SD was below the size of the graphic symbols.

Downloaded from http://mutage.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

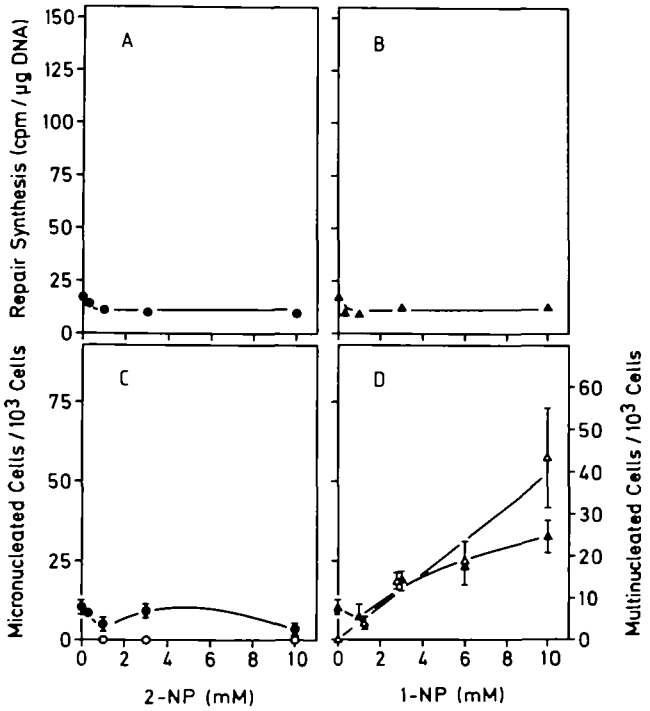

Fig. 4. DNA repair synthesis, micronuclei and multinucleated cells after exposure of V79 cells to 2-NP or 1-NP. V79 cells were exposed to the nitropropanes for either 5 h (DNA repair) or 3 h (micronuclei). Data are the means from two experiments (**A,B**) or the means ± SD of 3×500 cells from individual experiments (**C,D**). When no error bars are given, SD was below the size of the graphic symbols. ●,▲, repair synthesis (**A,B**) or micronucleated cells (**C,D**); ○,△, multinucleated cells (**C,D**).

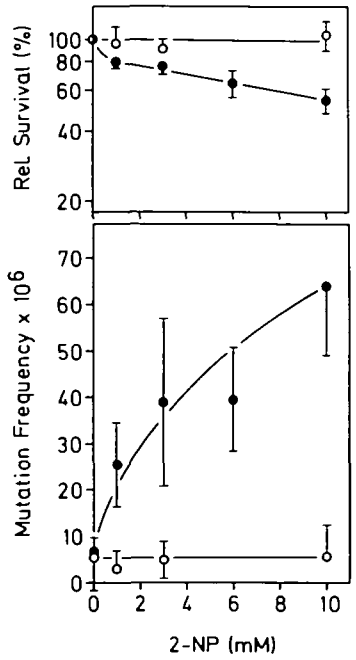

Fig. 5. Cytotoxicity and mutagenicity of 2-NP in H4IIEC3/G⁻ cells. Following pre-incubation of the cells in the presence (●,▲) or absence (○,△) of 2 µM DEX for 24 h, cells were exposed to 2-NP for another 24 h. Points represent the means ± SD of six plates from one of two experiments which yielded very similar results.

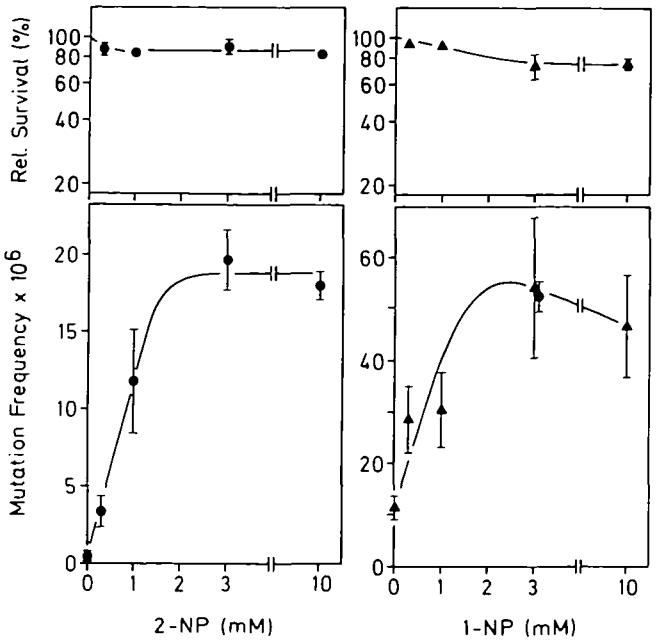

Fig. 6. Cytotoxicity and mutagenicity of 2-NP and 1-NP in V79 cells. Cells were exposed to 2-NP (●) and 1-NP (▲) for 3 h. Points represent the means ± SEM from three independent experiments on 2-NP and on 1-NP. When no error bars are given, SEM was below the size of the graphic symbols.

Table I. Effect of DEX on the activity of aldrin epoxidase in rat hepatoma cells[a]

Cell line	Aldrin epoxidase activity[b] (pmol/min/mg protein)	
	− DEX	+ DEX
C2Rev7	6.0 ± 0.2	22.3 ± 3.2
2sFou	9.1 ± 0.8	16.8 ± 1.3
H4IIEC3/G⁻	17.5 ± 1.1	16.8 ± 0.5

[a]Aldrin epoxidase was analysed after growing the cells in the presence or absence of 2 µM DEX for 24 h.
[b]Means ± SD of triplicate cultures.

Discussion

The present study was intended to help in clarifying the mechanisms which underly the genotoxicity of the carcinogen 2-NP in cultured mammalian cells. Several cell lines characterized for their expression of cytochrome P450-dependent monooxygenases were treated with 2-NP, and the induction of primary DNA damage and heritable genetic changes were measured. The experiments were performed in parallel with the non-carcinogenic isomer 1-NP. 2-NP was genotoxic in the hepatoma cell lines, an observation which is in agreement with our previous findings on the induction of DNA repair in rat liver cells *in vitro* and *in vivo* (Andrae *et al.*, 1988) as well as with the liver-specific carcinogenicity of the chemical (Fiala *et al.*, 1987). For a clear manifestation of the genotoxicity of 2-NP the hepatoma lines had to be pretreated with DEX, indicating that 2-NP is activated to genotoxic metabolites by a DEX-inducible enzymatic pathway. Cytochrome P450IA1 or other pathways related to the *Ah* receptor do not appear to be involved because C2Rev7 cells, in which both DNA repair and micronuclei are efficiently induced by 2-NP, do not contain detectable levels of this enzyme or its

Downloaded from http://mutage.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

mRNA (Wiebel *et al.*, 1984a–c; Corcos and Weiss, 1988) and lack the *Ah* receptor (P.Cikryt, personal communication). The liver specificity of the observed effects (Andrae *et al.*, 1988), and their enhancement after pretreatment of the cells with DEX, indicate that certain cytochrome P450II forms may be involved. This is supported by the observation that aldrin epoxidase, a marker function of certain liver-specific cytochrome P450 forms (Wolff and Guengerich, 1987), is present in the hepatoma cells, and also induced by DEX in C2Rev7 and 2sFou cells. The apparent lack of aldrin epoxidase induction in H4IIEC3/G$^-$ cells may be due to opposite alterations in specific P450 isozymes since P450 isozymes can be both increased and decreased by inducers (Mostafa *et al.*, 1981; Dawson *et al.*, 1985). This could result in an apparently unchanged activity of aldrin epoxidase, since aldrin epoxidation reflects the activity of several hepatic cytochrome P450 forms (Beaune *et al.*, 1986; Wolff and Guengerich, 1987). However, it is also possible that other DEX-inducible, cytochrome P450-independent metabolic routes are responsible for the induction of DNA damage resulting in DNA repair and micronuclei formation.

The results obtained following exposure of the V79 cells to 2-NP differed strikingly from the findings on the hepatoma cells. 2-NP was ineffective in inducing DNA repair and micronuclei but caused mutations to TG resistance. Mutagenicity was most likely caused by a mechanism different from that resulting in genotoxicity in hepatocytes or hepatoma cells because mutation induction (i) was not accompanied by increased repair synthesis or micronuclei formation and (ii) could not be further enhanced by DEX pretreatment of the cells. An involvement of cytochrome P450-dependent mono-oxygenases can be excluded as V79 cells lack these enzyme activities (Wiebel *et al.*, 1984c). Whether the compound is mutagenic *per se* in V79 cells, or whether it requires activation by some as yet unknown metabolic pathway, remains to be elucidated. Preliminary experiments suggest that nitroreduction, which has been shown to be responsible for part of the mutagenicity of 2-NP in *Salmonella typhimurium* (Göggelmann *et al.*, 1988), is not involved in the activation process (Andrae *et al.*, unpublished observations).

In contrast to 2-NP, 1-NP did not cause any detectable genotoxicity in the heptoma lines. The observation that the compound was not effective in inducing DNA repair synthesis in these cells is in line with our recent findings showing that 1-NP does not cause repair in rat hepatocytes *in vitro* or in rat liver *in vivo* (Andrae *et al.*, 1988). A lack of reactivity of 1-NP towards liver cell DNA is also indicated by the fact that the compound did not increase the frequency of micronuclei or mutations in the hepatoma cells. However, 1-NP clearly enhanced the formation of micronuclei in V79 cells, indicating that the effects of the compound in these cells differ from those in hepatic cells. Micronuclei formation in V79 cells was accompanied by a marked induction of multinucleated cells and TG resistant mutants, but not of DNA repair synthesis. Göggelmann *et al.* (1988) recently reported that 1-NP causes neither structural chromosomal aberrations nor sister chromatid exchanges in human lymphocytes. 1-NP is not mutagenic in *S.typhimurium* irrespective of the presence or absence of a metabolic activation system (Hite and Skeggs, 1979; Löfroth *et al.*, 1981; Speck *et al.*, 1982; Göggelmann *et al.*, 1988). The data suggest that exposure of V79 cells to 1-NP does not give rise to DNA modifications. It appears more likely that in these cells the induction of micronuclei and mutations by 1-NP is caused by an interaction of the compound or a metabolite with the mitotic apparatus of the cells, resulting in numerical chromosome aberrations. A similar mechanism has recently been suggested to explain the mutagencity of *o*-chlorobenzylidene malonitrile (Ziegler-Skylakakis *et al.*, 1989), a potent spindle poison (Schmid *et al.*, 1989) which does not react with DNA (Ziegler-Skylakakis *et al.*, 1989).

The reason for the different effects of 1-NP in hepatic cells and in the 'extra-hepatic' V79 cells is unclear. In the hepatoma lines 1-NP may be rapidly metabolized via enzymatic routes which are different from those prevailing in V79 cells and which prevent the induction of genotoxicity. Whether micronuclei and mutations in V79 cells are caused by 1-NP itself or by a metabolite remains to be established.

In conclusion, the results of this study suggest the existence of different pathways which may lead to genotoxic effects of nitropropanes in mammalian cells. One pathway is found in cells expressing liver-specific cytochrome P450 forms and is characterized by (i) inducibility by DEX, (ii) specificity for 2-NP and (iii) the formation of metabolites from 2-NP which elicit DNA repair synthesis, micronuclei and mutations. The other pathway(s) is found in V79 cells and is characterized by (i) independence of cytochrome P450, (ii) the induction of mutations by both 2-NP and 1-NP in the absence of any detectable effect on DNA repair synthesis, and (iii) the induction of micronucleated and multinucleated cells by 1-NP. The former, apparently liver cell-specific, pathway probably causes the formation of DNA lesions closely associated with the initiation of liver tumours in the animal. The relevance for tumour induction of the latter pathway, which occurs in cells of extra-hepatic origin, is still unclear.

Acknowledgement

The expert technical assistance of Ms Heike Hornfeldt is gratefully acknowledged.

References

Andrae,U., Hornfeldt,H., Vogl,L., Lichtmannegger,J. and Summer,K.H. (1988) 2-Nitropropane induces DNA repair synthesis in rat hepatocytes *in vitro* and *in vivo*. *Carcinogenesis*, **9**, 811–815.

Beaune,P.H., Kremers,P.G., Kaminsky,L.S., De Graeve,J., Albert, A. and Guengerich,F.P. (1986). Comparison of monooxygenase activities and cytochrome P-450 isozyme concentrations in human liver microsomes. *Drug Metab. Dispos.*, **14**, 437–442.

Corcos,L. and Weiss,M.C. (1988) Phenobarbital, dexamethasone and benzanthracene induce several cytochrome P450 mRNAs in rat hepatoma cells. *FEBS Lett.*, **233**, 37–40.

Dawson,J.R., Adams,D.J. and Wolf,C.R. (1985) Induction of drug metabolizing enzymes in human liver cell line Hep G2. *FEBS Lett.*, **182**, 219–222.

Fiala,E.S., Czerniak,R., Castonguay,A., Conaway,C.C. and Rivenson,A. (1987) Assay of 1-nitropropane, 2-nitropropane, 1-azoxypropane and 2-azoxypropane for carcinogenicity by gavage in Sprague-Dawley rats. *Carcinogenesis*, **8**, 1947–1949.

Göggelmann,W., Bauchinger,M., Kulka,U. and Schmid,E. (1988) Genotoxicity of 2-nitropropane and 1-nitropropane in *Salmonella typhimurium* and human lymphocytes. *Mutagenesis*, **3**, 137–140.

Griffin,T.B., Coulston,F. and Stein,A.A. (1980) Chronic inhalation exposure of rats to vapors of 2-nitropropane at 25 p.p.m. *Ecotoxicol. Environ. Safety*, **4**, 267–281.

Griffin,T.B., Stein,A.A. and Coulston,F. (1982) Inhalation exposure of rats to vapors of 1-nitropropane at 100 p.p.m. *Ecotoxicol. Environ. Safety*, **6**, 268–282.

Hadidian,Z., Fredrickson,T.N., Weisburger,E.K., Weisburger,J.H., Glass,R.M. and Manlet,N. (1968) Tests for chemical carcinogens. Report on the activity of aromatic amines, nitrosamines, quinolines, amides, epoxides, aziridines and purine antimetabolites. *J. Natl. Cancer Inst.*, **41**, 985–1025.

Hite,M. and Skeggs,H. (1979) Mutagenic evaluation of nitroparaffins in the *Salmonella typhimurium*/mammalian-microsome test and the micronucleus test. *Environ. Mutagenesis*, **1**, 383–389.

Lewis,T.R., Ulrich,C.E. and Busey,W.M. (1979) Subchronic inhalation toxicity of nitromethane and 2-nitropropane. *J. Environ. Pathol. Toxicol.*, **2**, 233–249.

Löfroth,G., Nilsson,L. and Andersen,J.R. (1981) The mutagenicity of nitroalkanes in the Ames *Salmonella*/microsome assay. *Environ. Mutagenesis*, **3**, 336–337.

Downloaded from http://mutage.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016

Mostafa,M.H., Ruchirawat,M. and Weisburger,E.K. (1981) Comparative studies on the effects of various microsomal enzyme inducers on the *N*-demethylation of dimethylnitrosamine. *Biochem. Pharmacol.*, **30**, 2007–2011.

Nebert,D.W., Nelson,D.R., Adesnik,M., Coon,M.J., Estabrook,R.W., Gonzalez,F.J., Guengerich,F.P., Gunsalus,I.C., Johnson,E.F., Kemper,B., Levin,W., Phillips,I.R., Sato,R. and Waterman,M.R. (1989) The P450 superfamily: updated listing of all genes and recommended nomenclature for the chromosomal loci. *DNA*, **8**, 1–13.

Pettijohn,D.E. and Hanawalt,P.C. (1964) Evidence of repair replication of ultraviolet damaged DNA in bacteria. *J. Mol. Biol.*, **9**, 359–410.

Pitot,H.C., Peraino,C., Morse,P.A. and Potter,V. (1964) Hepatomas in tissue culture compared with adapting liver *in vivo*. *NCI Monograph*, **13**, 229–245.

Reuber,M.D. (1961) A transplantable bile secreting hepatocellular carcinoma in the rat. *J. Natl. Cancer Inst.*, **26**, 891–899.

Roscher,E. and Wiebel,F.J. (1988) Mutagenicity, clastogenicity and cytotoxicity of procarcinogens in a rat hepatoma cell line competent for xenobiotic metabolism. *Mutagenesis*, **3**, 269–276.

Rossberger,S. and Andrae,U. (1985) DNA repair synthesis induced by *N*-hydroxyurea, acetohydroxamic acid, and *N*-hydroxyurethane in primary hepatocyte cultures: comparative evaluation using the autoradiographic and the bromodeoxyuridine density-shift method. *Mutat. Res.*, **145**, 201–207.

Schmid,E., Bauchinger,M., Ziegler-Skylakakis,K. and Andrae,U. (1989) 2-Chlorobenzylidene malonitrile (CS) causes spindle disturbances in V79 Chinese hamster cells. *Mutat. Res.*, **226**, 133–136.

Smith,C.A. and Hanawalt,P.C. (1976) Repair replication in human cells; simplified determination utilizing hydroxyurea. *Biochim. Biophys. Acta*, **432**, 336–347.

Speck,W.T., Meyer,L.W., Zeiger,E. and Rosenkranz,H.S. (1982) Mutagenicity and DNA-modifying activity of 2-nitropropane. *Mutat. Res.*, **104**, 49–54.

Wiebel,F.J., Park,S.S., Kiefer,F. and Gelboin,H.V. (1984a) Expression of cytochromes P-450 in rat hepatoma cells. Analysis by monoclonal antibodies specific for cytochromes P-450 from rat liver induced by 3-methylcholanthrene or phenobarbital. *Eur. J. Biochem.*, **145**, 455–462.

Wiebel,F.J., Kiefer,F. and Murdia,U.S. (1984b) Phenobarbital induces cytochrome P-450 and cytochrome P-448-dependent monooxygenases in rat hepatoma cells. *Chem.-Biol. Interact.*, **52**, 151–162.

Wiebel,F.J., Lambiotte,M., Singh,J., Summer,K.H. and Wolff,T. (1984c) Expression of carcinogen-metabolizing enzymes in continuous cultures of mammalian cells. In Greim,H., Jung,R., Kramer,M., Marquardt,H. and Oesch,F. (eds), *Biochemical Basis of Chemical Carcinogenesis*. Raven Press, New York, pp. 77–88.

Wolff,T. and Guengerich,F.P. (1987) Rat liver cytochrome P-450 isozymes as catalysts of aldrin epoxidation in reconstituted monooxygenase systems and microsomes. *Biochem. Pharmacol.*, **36**, 2581–2588.

Wolff,T., Deml,E. and Wanders,H. (1979) Aldrin epoxidation, a highly sensitive indicator specific for cytochrome P-450-dependent monooxygenase activities. *Drug. Metab. Dispos.*, **7**, 301–305.

Ziegler-Skylakakis,K., Summer,K.H. and Andrae,U. (1989) Mutagenicity and cytotoxicity of 2-chlorobenzylidene malonitrile (CS) and metabolites in V79 Chinese hamster cells. *Arch. Toxicol.*, **63**, 314–319.

Received on November 20, 1989; accepted on February 14, 1990

Downloaded from http://mutage.oxfordjournals.org/ at Pennsylvania State University on March 5, 2016