Carcinogenesis vol.9 no.5 pp.811–815, 1988

2-Nitropropane induces DNA repair synthesis in rat hepatocytes *in vitro* and *in vivo*

U.Andrae[1], H.Homfeldt, L.Vogl, J.Lichtmannegger and K.H.Summer

Institut für Toxikologie, Gesellschaft für Strahlen- und Umweltforschung (GSF), D-8042 Neuherberg/München, FRG

[1]To whom correspondence should be addressed

The genotoxicity of 2-nitropropane (2-NP) and 1-nitropropane (1-NP) was investigated by measuring the induction of DNA repair synthesis in rat liver cells *in vitro* and *in vivo*. 2-NP strongly induced DNA repair synthesis in both cases. When applied *in vivo*, 2-NP was considerably more effective in hepatocytes from males than in those from females. 1-NP was not active *in vitro* or *in vivo*. 2-NP and 1-NP did not induce repair in cell lines of extrahepatic origin derived from rat, mouse, hamster and man. The results are consistent with the reported carcinogenicity of 2-NP in rat liver and suggest that the formation of hepatocarcinomas by 2-NP is due to the generation of a genotoxic metabolite from 2-NP by liver-specific metabolism.

Introduction

2-Nitropropane (2-NP*), a widely used industrial solvent, induces liver tumours in rats following prolonged inhalation (1,2), males being more sensitive than females (2). The mechanism of tumour induction is still unclear. Genotoxic and epigenetic mechanisms have been considered. Ullrich *et al.* (3) showed that liver microsomes from rats treated with phenobarbital or methylcholanthrene catalyse the oxidative denitrification of 2-NP. They speculated that the nitrite formed reacts with endogenous amines to yield carcinogenic nitrosamines. Speck *et al.* (4) suggested that 2-NP might be a direct-acting genotoxic agent since DNA damage was induced in isolated DNA following incubation with 2-NP at 56°C for 18 h. This view was supported by results obtained by the Ames test which showed that the mutagenicity of 2-NP can be observed in the absence of an activating fraction (S9) from rat liver (4–6) and that mutagenicity is not significantly altered in the presence of S9 (4,5). Griffin *et al.* (2) claimed the hepatocarcinogenicity of 2-NP not to be due to the genotoxicity of the compound but to be the consequence of cytotoxic effects on the liver which are observed after exposure of the animals to high doses of 2-NP. According to these authors massive cell damage occurs in the liver and the subsequent cell regeneration eventually leads to tumour formation. Two independent micronucleus assays in bone marrow of mice treated with 2-NP yielded negative results (5,7).

In the present study we investigated whether 2-NP causes DNA damage in mammalian cells by determining the effects of the compound in its target organ, the liver. Genotoxicity was monitored by measuring the induction of DNA repair synthesis in primary rat hepatocyte cultures (HPC) following exposure of the cells to the compound *in vitro* and following treatment of the animals *in vivo*. Repair was measured in cells from both male and female rats to determine whether the differences between the sexes in their susceptibility to tumour induction (2) were reflected by differences in the genotoxicity of 2-NP. In order to investigate the possibility that the action of 2-NP is organ specific we also determined the capacity of 2-NP to elicit DNA repair synthesis in a variety of continuous mammalian cell lines derived from different organs. The effects of 2-NP on hepatocytes were compared to those of its structural analogue, 1-nitropropane (1-NP). In contrast to 2-NP, 1-NP is not mutagenic in Salmonella in either the presence or absence of metabolic activation (4–6). It is not known whether the compound is carcinogenic.

*Abbreviations: BME, basal medium Earle; BrdUrd, 5-bromodeoxyuridine; dCyd, deoxycytidine; dThd, thymidine; DMN, dimethylnitrosamine; FdUrd, 5-fluorodeoxyuridine; HPC, hepatocyte primary cultures; HU, hydroxyurea; MMS, methyl methanesulfonate; 1-NP, 1-nitropropane; 2-NP, 2-nitropropane; WME, William's medium E.

Materials and methods

Chemicals

2-NP (>99% pure) was a gift from Angus Chemie, Ibbenbüren-Uffeln. 1-NP was obtained from Merck, Darmstadt and purified by distillation (final purity 97.4%, major impurity 2.3% 2-NP). Dimethylnitrosamine (DMN) and methyl methanesulfonate (MMS) were purchased from Merck-Schuchardt, Hohenbrunn; 5-bromodeoxyuridine (BrdUrd) and 5-fluorodeoxyuridine (FdUrd) from Sigma, München, William's medium E (WME) from Flow, Meckenheim; fetal calf serum from Gibco, Karlsruhe; collagenase and proteinase K from Boehringer, Mannheim: CsCl (analytical grade) from Serva, Heidelberg, Cs_2SO_4 (suprapur) from Merck, Darmstadt; [5-^{3}H]deoxycytidine ([^{3}H]dCyd, 18 Ci/mmol) and [methyl-^{3}H]thymidine ([^{3}H]dThd, 44 Ci/mmol) from Amersham, Braunschweig.

Animals

Male and female Wistar rats (inbred strain, Neuherberg) weighing 150–200 g were fed a standard laboratory diet (Altromin, Lage) and had free access to tap water.

Cells

Hepatocytes were prepared by perfusion of the liver with collagenase according to the method of Berry and Friend (8) except that no hyaluronidase was used (9). Viability of the cells was routinely determined by staining with trypan blue. Hepatocyte preparations were used only when >80% of the cells excluded the dye.

The continuous cell lines were kindly supplied by Dr F.J.Wiebel, GSF, Neuherberg.

Measurement of DNA repair synthesis

Repair in HPC treated in vitro. Repair synthesis in HPC was determined using the BrdUrd density-shift method as described previously (10). 6×10^6 viable cells were allowed to attach in WME + 10% fetal calf serum ('complete WME') in 75-cm^2 flasks for 1.5 h. Attached cells were preincubated in complete WME containing 200 μM BrdUrd and 40 μM FdUrd for 1 h. After preincubation the medium was changed and the incubation continued in serum-free WME with BrdUrd, FdUrd, [^{3}H]dCyd (10 μCi/ml) in the presence or absence of a test compound for 18–20 h. 1-NP and 2-NP were dissolved in medium immediately before each experiment. To avoid evaporation of the nitropropanes during incubation, flasks were closed air-tight. All operations were carried out under subdued (golden) light. Following incubation, the DNA was isolated and centrifuged to equilibrium in alkaline CsCl/Cs_2SO_4 density gradients (Beckman rotor VTi65, 48 000 r.p.m., 20°C, 16 h). Parental ('light') DNA strands were rebanded in a second alkaline gradient. Repair replication was determined from the gradient profiles as radioactivity incorporated into parental DNA and expressed as c.p.m./μg DNA.

Repair in HPC following in vivo treatment. Rats were injected i.p. with the compounds dissolved in olive oil (2-NP, 1-NP, MMS) or water (DMN) to give a final injection volume of 2 ml/kg body weight. The animals were killed after various time periods and HPC were prepared. Prelabelling of the HPC with BrdUrd

and FdUrd was performed during the 1.5-h attachment period in complete WME. Subsequently, cells were washed with prewarmed Hank's solution and the incubation was continued in serum-free WME containing BrdUrd, FdUrd and [^{3}H]dCyd (10 μCi/ml) for 18–20 h. Following incubation, the DNA was isolated and repair synthesis determined as described above.

Repair in continuous cell lines. Cells grown in 75-cm^2 flasks were preincubated in basal medium Earle (BME) containing 10 μM BrdUrd and 2 μM FdUrd for 1 h. After preincubation the medium was changed and cells were incubated in BME with BrdUrd, FdUrd, 2 mM hydroxyurea (HU) and [^{3}H]dThd (10 μCi/ml) in the presence or absence of the test compound for another 5 h. Then the DNA was isolated and repair synthesis determined as described above.

Results

Induction of repair synthesis in HPC by treatment of the cells with 2-NP and 1-NP

HPC were exposed to various concentrations of 2-NP in the presence of BrdUrd, FdUrd and 10 μCi [^{3}H]dCyd/ml for 18–20 h. The DNA was isolated and analysed for the induction of DNA repair synthesis by centrifugation in alkaline $CsCl/Cs_2SO_4$ density gradients. 2-NP markedly increased repair incorporation in HPC from both males (Figure 1) and females (Figure 2). Repair induction was detectable at concentrations as low as 30 μM (~2-fold increase above controls). In HPC from male rats, repair synthesis increased linearly up to a 2-NP concentration of ~0.5 mM and then levelled off abruptly (Figure 1). The maximum repair incorporation obtained (12- to 15-fold increase above control) was similar to that induced by 10 mM DMN which served as positive control (Figure 1). In HPC from females (Figure 2) 2-NP-induced repair increased up to a concentration of ~10 mM. At this concentration repair incorporation was increased 25- to 30-fold above the control and ~2-fold above the value obtained with 10 mM DMN.

Exposure of HPC from male and female rats to 1-NP (0.1–10 mM) resulted in an up to 5-fold increase in repair incorporation (data not shown). However, this repair induction can be completely attributed to 2-NP present as an impurity of 2.3% in the 1-NP charge used, strongly suggesting that 1-NP itself was inactive.

Induction of repair synthesis in HPC following treatment of animals with 2-NP and 1-NP

In order to start radioactive labelling of repair synthesis as early as possible following sacrifice of the exposed animals, prelabelling of the cells with BrdUrd and FdUrd was performed during the 1.5-h attachment period. Under these conditions repair synthesis in cultures from untreated males amounted to 95.3 ± 13.8 c.p.m./μg DNA (mean ± SD from nine animals). This was ~30% higher than the values obtained in the *in vitro* experiments involving separate attachment and preincubation periods (73.3 ± 4.9 c.p.m./μg DNA, mean ± SD from five experiments). Repair incorporation in HPC derived from untreated female rats was lower and did not differ significantly from the repair value obtained in the *in vitro* experiments [59.8 ± 7.0 c.p.m./μg DNA (mean ± SD from three animals) versus 61.8 c.p.m./μg DNA (mean from two animals)].

A strong induction of repair synthesis was observed in HPC from male rats which had been treated with 2-NP *in vivo* for 4 h (Figure 3). At 20 mg 2-NP/kg, the lowest dose applied, repair incorporation was about doubled and at 80 mg/kg it was increased ~3.6-fold. 2-NP was also genotoxic in HPC from female rats treated *in vivo* (Figure 4). However, repair induction was less pronounced than in HPC from male rats. It increased slowly between 20 and 40 mg 2-NP/kg and more rapidly at higher concentrations.

1-NP did not cause increased repair synthesis in males treated with 20–80 mg/kg for 4 h but did slightly reduce the repair background (Figure 3). Likewise, no repair induction was observed when male rats were injected with 60 mg 1-NP/kg and killed 1 h or 17 h later (Table I). 1-NP was also ineffective in inducing repair in HPC from female rats treated *in vivo* (Figure 4).

The induction of repair synthesis by 2-NP *in vivo* was compared with the repair observed after treatment of the rats with DMN or MMS, carcinogens which have been shown to induce DNA repair in HPC following *in vivo* treatment (11–13). Table

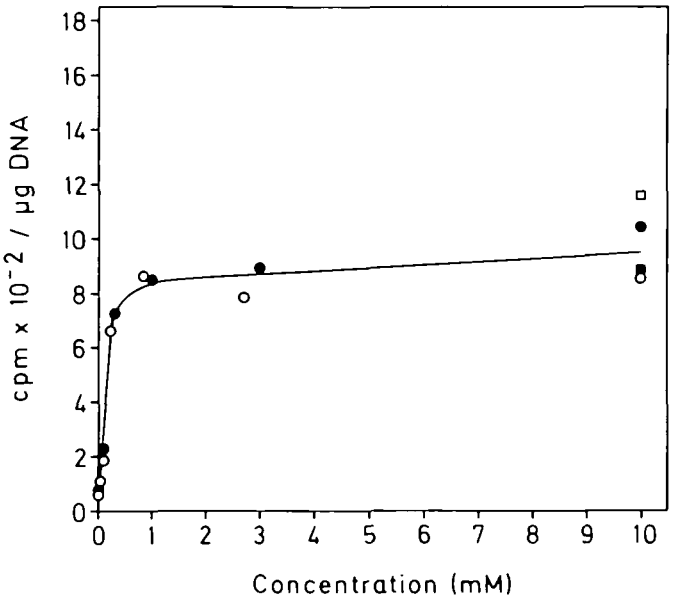

Fig. 1. Induction of DNA repair synthesis in HPC from male rats by 2-NP and DMN *in vitro*. Following an attachment period of 1.5 h cells were preincubated with FdUrd and BrdUrd for 1 h. Subsequently HPC were exposed to 2-NP or DMN in the presence of FdUrd, BrdUrd and [^{3}H]dCyd (10 μCi/ml) for 20 h. Repair synthesis was determined as described in Materials and methods. ●, ○, 2-NP; ■,□. DMN. Data from two independent experiments (closed and open symbols) are shown.

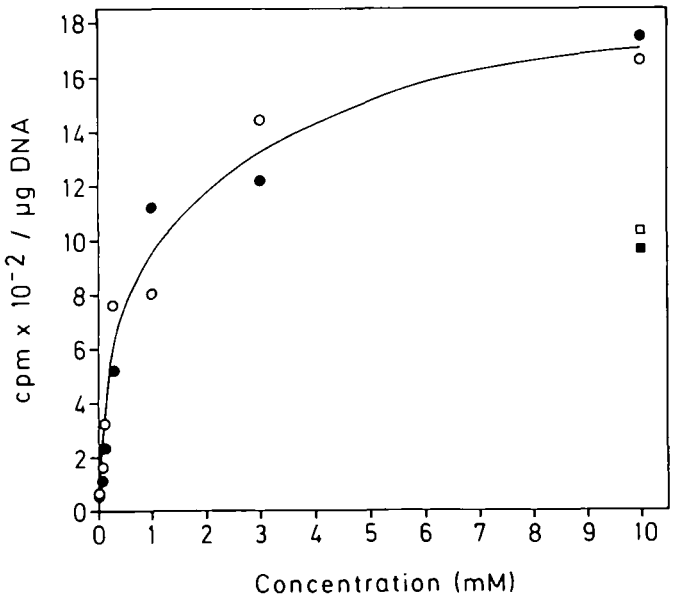

Fig. 2. Induction of DNA repair synthesis in HPC from female rats by 2-NP and DMN *in vitro*. See legend to Figure 1.

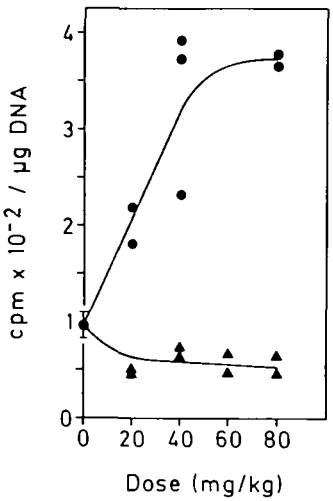

Fig. 3. Induction of DNA repair synthesis in HPC from male rats treated with 2-NP or 1-NP *in vivo*. Animals were injected i.p. with 2-NP or 1-NP and killed 4 h later. Hepatocytes were isolated and allowed to attach in the presence of FdUrd and BrdUrd for 1.5 h. Subsequently HPC were exposed to [^{3}H]dCyd (10 µCi/ml), FdUrd and BrdUrd for 20 h. Repair synthesis was determined as described in Materials and methods. Each data point represents the results from one animal. ●, 2-NP; ▲, 1-NP.

Table I. Repair synthesis in HPC derived from male rats treated with 60 mg 1-NP/kg

Treatment	Repair synthesis (c.p.m./µg DNA)[a]
Control	95.3 ± 13.8 (n = 9)[b]
1 h	115.5 ± 12.2 (n = 2)
17 h	106.8 ± 3.9 (n = 2)

[a]Mean ± SD (n = 9) or mean ± range (n = 2).
[b]Number of animals.

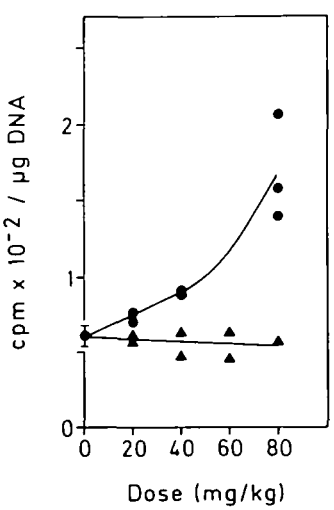

Fig. 4. Induction of DNA repair synthesis in HPC from female rats treated with 1-NP or 2-NP *in vivo*. See legend to Figure 3.

II shows the results obtained with male and female rats exposed to 10 mg DMN/kg and 100 mg MMS/kg for 2 h. DMN was a much better inducer of DNA repair than 2-NP in both males and females, whereas MMS was much less potent than 2-NP in males and similarly active in females.

Table II. Repair synthesis in HPC derived from male and female rats treated with 2-NP, DMN or MMS[a]

Compound (dose)	Repair synthesis (c.p.m./µg DNA)[b]			
	Males	(n)	Females	(n)
Control	95.3 ± 13.8	(6)	59.8 ± 7.0	(3)
2-NP (80 mg/kg)	370.8 ± 6.8	(2)	167.7 ± 34.6	(3)
DMN (10 mg/kg)	698.2 ± 9.2	(2)	480.2 ± 13.9	(2)
MMS (100 mg/kg)	199.6 ± 16.9	(2)	186.1 ± 15.2	(2)

[a]Animals were killed 4 h (2-NP) or 2 h (DMN, MMS) following i.p. injection of the test compound.
[b]Mean ± SD (n = 3) or mean ± range (n = 2).

Table III. DNA repair synthesis in established cell lines from man, mouse, hamster and rat exposed to 2-NP, 1-NP or MMS[a]

Cell line	Repair synthesis[b]		
	2-NP	1-NP	MMS
Human cells			
WI38 (embryonic fibroblasts, lung)	1[c]	1	10
NCI-H322 (adenocarcinoma, lung)	1	1	123
A549 (adenocarcinoma, lung)	1	1	15
HEp2 (epiderm. carcinoma, larynx)	1	1	18
Mouse			
3T3-NIH (embryonic fibroblasts)	1	1	156
Hamster cells			
V79 (fibroblasts, lung)	1	1	47
CHO (fibroblasts, ovary)	1	1	122
Rat cells			
208 F (embryonic fibroblasts)	1	1	32
LLC-WRC 256 (carcinoma, Walker rat)	1	1	69
Hepatocytes (positive control)	28	ND[d]	18

[a]Cell lines were preincubated with FdUrd and BrdUrd for 1 h. Subsequently, they were exposed to 2-NP (10 mM), 1-NP (10 mM) or MMS (1 mM) in the presence of FdUrd, BrdUrd, HU and [^{3}H]dThd (10 µCi/ml) for 5 h. Incubation of HPC was performed as described in Materials and methods.
[b]Factor relative to the untreated control.
[c]Increase in repair synthesis <20% in comparison to the untreated control.
[d]Not done.

Induction of repair synthesis in mammalian cell lines

Cells were incubated with 2-NP or 1-NP (1 − 10 mM) in the presence of HU, FdUrd, BrdUrd and [^{3}H]dThd for 5 h and then analysed for the induction of repair as described above. In these experiments, HU (2 mM) was used to suppress semiconservative DNA synthesis thus facilitating the determination of repair synthesis in these rapidly proliferating cell lines (14). Whereas MMS, which served as positive control, strongly increased repair synthesis in all of the cell lines examined, both 2-NP and 1-NP were completely ineffective (Table III). The lack of genotoxicity of 2-NP in the cell lines was not simply a consequence of the relatively short incubation time since strong repair induction was observed when HPC were incubated for the same length of time with 10 mM 2-NP (Table III).

Discussion

In the present study we examined the genotoxicity of 2-NP and 1-NP in rat HPC *in vitro* and following treatment of the animals *in vivo*. Genotoxicity was measured as induced repair replica-

tion by means of a modification (10,15) of the BrdUrd density-shift method (16). This method has not been applied previously to the measurement of repair induced in hepatocytes *in vivo*. In order to start radioactive labelling of repair in HPC from rats treated *in vivo* as early as possible following sacrifice of the animals the protocol for measuring repair (10) was modified. Instead of prelabelling the cells with BrdUrd and FdUrd after the 1.5-h attachment period, cells were allowed to attach in the presence of BrdUrd and FdUrd. Using this modification, radioactive labelling of DNA repair synthesis began 1 h earlier after liver perfusion than in the experiments in which HPC were exposed to the test compounds *in vitro*. Comparison of the respective repair values obtained in the absence of any test compound shows that in HPC from males the 'background repair' was ~30% higher when the *in vivo* protocol was applied. This is most likely to result from a higher proportion of repair of collagenase-induced DNA damage (15) being measured under these conditions. This interpretation is supported by the time-course of the repair of collagenase-induced DNA strand breaks which is most rapid immediately after cell isolation (17), and by the observation that the repair background is markedly reduced when repair labelling of HPC is started 12 h after liver perfusion (Rossberger and Andrae, unpublished observations). In HPC derived from female rats no difference was observed between the control values obtained by the *in vitro* and the *in vivo* protocol. The reason for the different behaviour of hepatocytes from males and females is not yet clear.

The results show that 2-NP efficiently induces DNA repair synthesis in hepatocytes both *in vitro* and *in vivo*, whereas 1-NP is inactive under both conditions. In order to determine the *in vivo* effects of 2-NP and 1-NP, animals were dosed by i.p. injection. It has been demonstrated that absorption of 2-NP by the lung is only limited by the ventilation rate and that the compound accumulates in the animals during inhalation (J.Filser and B.Denk, personal communication). Unmetabolized 2-NP appears in the blood (18) as is the case after i.p. administration. Therefore, the latter is considered to be a useful application route although, for the human situation, inhalation is thought to be the main route of exposure.

The lack of genotoxicity of 2-NP and 1-NP in the many mammalian cell lines examined strongly suggests that 2-NP is not a direct-acting genotoxic agent but requires metabolic activation. At present, the metabolic route leading to the formation of DNA damaging metabolites is not known. Both oxidative and reductive activation mechanisms appear possible. Metabolism of 2-NP in rat results primarily in the formation of acetone, isopropanol and CO_2 (18) suggesting oxidative denitrification as the major metabolic pathway *in vivo*. Likewise, Ullrich *et al.* (3) showed that liver microsomes from rats treated with 3-methylcholanthrene or phenobarbital catalyse the formation of nitrite and acetone from 2-NP and, to a lesser extent, 1-NP. Their observations suggested that microsomal denitrification is dependent on cytochrome P-450. Recently the hypothesis has been put forward that radical intermediates may be produced in the course of this reaction (19). Microsomes from untreated rats, however, did not catalyse nitrite formation from 2-NP to any significant extent, a finding which calls into question the role of hepatic mono-oxygenases in oxidative denitrification in the uninduced rat. A number of experiments have shown that 2-NP can be oxidatively denitrificated *in vitro* by various enzymes other than cytochrome P-450, such as horseradish peroxidase (19–22), various oxidases (23,24) or certain microbial flavoprotein dioxygenases (25,26). Formation of 2-NP radicals (19,27,28) and reactive forms of oxygen (19,21, 27–30) has been demonstrated for some of these reactions. The possibility that similar reactions occur in intact mammalian cells has not yet been investigated.

Another conceivable pathway for oxidative metabolism of 2-NP in liver is oxidation of the C1 of the carbon chain with preservation of the nitro group. The question of the occurrence of this metabolic route, and its possible toxicological implications, has as yet received little attention.

2-NP is mutagenic in *Salmonella typhimurium* in the absence of hepatic S9 mix (4–6). This result indicates that cytochrome P-450 is not necessary for metabolic activation since Salmonella cells lack detectable mono-oxygenase activities. In this case other bacterial enzymes must be involved in the production of mutagenic products from 2-NP. Conceivably, metabolic activation may occur via enzymatic reduction of the nitro group in analogy to the activation of aromatic nitro compounds. Although the mutagenicity of 2-NP in Salmonella was the same in wild-type and mutants deficient in the 'classical' nitroreductase (NR^-) (4) this does not necessarily exclude nitroreduction as an activation mechanism. Other yet unidentified nitroreductases could be involved in reductive metabolism of 2-NP in analogy to some of the dinitropyrenes which are reductively activated in Salmonella by an enzyme different to the 'classical' nitroreductase (31). At present it is open to question whether there is any similarity between the mechanisms leading to the induction of gene mutations in bacteria and to the induction of DNA repair in hepatocytes or whether different activation pathways cause the genetic effects observed in the two cell types.

An intriguing finding of the present investigation is the marked difference in the susceptibilities of hepatocytes from male and female animals to the induction of DNA repair by 2-NP. Curiously, repair induction *in vitro* was much higher in cells from female than from male rats, whereas it was the other way round *in vivo*. The reason for this discrepancy is not clear at present.

In hepatocytes from male rats treated with 2-NP *in vitro* repair incorporation abruptly levelled off at concentrations higher than ~0.5 mM. The result suggests that either the production of DNA-damaging metabolites from 2-NP, or the repair of the resulting DNA lesions, becomes saturated. The capacity of the cells for repair incorporation of [^{3}H]dCyd is not limiting since considerably more repair incorporation can be induced in these cells, e.g. by treatment with 50 mM DMN (not shown). A detailed analysis of the results obtained in the *in vivo* experiments is difficult in view of the small numbers of animals (two or three) used for each dose. Nevertheless, the data suggest that a similar saturation phenomenon occurs in hepatocytes from male rats exposed *in vivo* to 2-NP doses >60 mg/kg, although such doses do not saturate the capacity of the cells for repair synthesis as shown by the much higher repair incorporation obtained after treatment of the animals with 10 mg DMN/kg. Thus the most plausible explanation for these saturation effects is an exhaustion of the metabolic pathway leading to the formation of DNA lesions. No saturation effect was observed in hepatocytes from female rats treated *in vivo* with 2-NP doses up to 80 mg/kg. On the contrary, DNA damage was produced relatively more efficiently at higher doses than at lower doses. These data suggest that some detoxication pathway may exist in female rats which becomes saturated at 2-NP doses higher than ~50 mg/kg.

The difference in the capacity of 2-NP to induce repair synthesis in hepatocytes from male and female rats corresponds well to its distinctly different carcinogenic potency in male and female liver (2) and gives additional support to the idea that the induction of liver tumours by 2-NP is a consequence of genotoxicity.

References

1. 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.
2. 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.
3. Ullrich,V., Hermann,G. and Weber,P. (1978) Nitrite formation from 2-nitropropane by microsomal monooxygenases. *Biochem. Pharmacol.*, **27**, 2301–2304.
4. 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.
5. 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.
6. Löfroth,G., Nilsson,L. and Andersen,J.R. (1981) The mutagenicity of nitroalkanes in the Ames *Salmonella*/microsome assay. *Environ. Mutagenesis*, **3**, 336–337.
7. Kliesch,U. and Adler,I.-D. (1987) Micronucleus test in bone marrow of mice treated with 1-nitropropane, 2-nitropropane and cisplatin. *Mutat. Res.*, **192**, 181–184.
8. Berry,M.N. and Friend,D.S. (1969) High-yield preparation of isolated rat liver parenchymal cells. A biochemical and fine structural study. *J. Cell Biol.*, **43**, 506–520.
9. Sies,H. and Summer,K.-H. (1975) Hydroperoxide metabolizing systems in rat liver *Eur. J. Biochem.*, **57**, 503–512.
10. Rossberger,S. and Andrae,U. (1985) DNA repair synthesis induced by N-hydroxyurea, acetohydroxamic acid, and *N*-hydroxyurethane in primary rat hepatocyte cultures: comparative evaluation using the autoradiographic and the bromodeoxyuridine density-shift method. *Mutat. Res.*, **145**, 201–207.
11. Mirsalis,J.C. and Butterworth,B.E. (1980) Detection of unscheduled DNA synthesis in hepatocytes isolated from rats treated with genotoxic agents: an *in vivo–in vitro* assay for potential carcinogens and mutagens. *Carcinogenesis*, **1**, 621–625.
12. Mirsalis,J.C., Tyson,C.K. and Butterworth,B.E. (1982) Detection of genotoxic carcinogens in the *in vivo–in vitro* hepatocyte DNA repair assay. *Environ. Mutagenesis*, **4**, 553–562.
13. Mirsalis,J.C., Tyson,C K., Loh,E.N., Steinmetz,K.L., Bakke,J.P., Hamilton,C.M., Spak,D.K. and Spalding,J.W. (1985) Induction of hepatic cell proliferation and unscheduled DNA synthesis in mouse hepatocytes following *in vivo* treatment. *Carcinogenesis*, **6**, 1521–1524.
14. Smith,C.A. and Hanawalt,P.C. (1976) Repair replication in human cells; simplified determination utilizing hydroxyurea. *Biochim. Biophys. Acta*, **432**, 336–347.
15. Rossberger,S. and Andrae,U. (1987) Background DNA repair synthesis in rat hepatocyte cultures used for genotoxicity testing. *Toxicol. In Vitro*, **1**, 215–223.
16. Pettijohn,D.E. and Hanawalt,P.C. (1964) Evidence of repair replication of ultraviolet damaged DNA in bacteria. *J. Mol. Biol.*, **9**, 395–410.
17. Cesarone,C.F., Fugassa,E., Gallo,G., Voci,A. and Orunesu,M. (1984) Collagenase perfusion of rat liver induces DNA damage and DNA repair in hepatocytes. *Mutat. Res.*, **141**, 113–116.
18. Müller,W.F., Coulston,F. and Korte,F. (1983) Comparative metabolism of 2-nitropropane in rats and chimpanzees. *Chemosphere*, **12**, 231–237.
19. Porter,D.J.T. and Bright,H.J. (1983) The mechanism of oxidation of nitroalkanes by horseradish peroxidase. *J. Biol. Chem.*, **258**, 9913–9924.
20. Little,H.N. (1957) The oxidation of 2-nitropropane by extracts of pea plants. *J. Biol. Chem.*, **229**, 231–238.
21. DeRycker,J. and Halliwell,B. (1978) Oxidation of 2-nitropropane by horseradish peroxidase. Involvement of hydrogen peroxide and of superoxide in the reaction mechanism. *Biochem. J.*, **175**, 601–606.
22. Indig,G.L. and Cilento,G. (1987) Peroxidase-promoted aerobic oxidation of 2-nitropropane: mechanism of excited state formation. *Biochim. Biophys. Acta*, **923**, 347–354.
23. Kido,T., Hashizume,K. and Soda,K. (1978) Purification and properties of nitroalkane oxidase from *Fusarium oxysporum*. *J. Bacteriol.*, **133**, 53–58.
24. Dhawale,M.R. and Hornemann,U. (1979) Nitroalkane oxidation by *Streptomycetes*. *J. Bacteriol.*, **137**, 916–924.
25. Kido,T., Soda,K., Suzuki,T. and Asada,K. (1976) A new oxygenase, 2-nitropropane dioxygenase of *Hansenula mrakii*. *J. Biol. Chem.*, **251**, 6994–7000.
26. Kido,T., Yamamoto,T. and Soda,K. (1976) Purification and properties of nitroalkane-oxidizing enzyme from *Hansenula mrakii*. *J. Bacteriol.*, **126**, 1261–1265.
27. Kuo,C.F. and Fridovich,I. (1986) Free-radical chain oxidation of 2-nitropropane initiated and propagated by superoxide. *Biochem. J.*, **237**, 505–510.
28. Fiala,E.S., Conaway,C.C., Biles,W.T. and Johnson,B. (1987) Enhanced mutagenicity of 2-nitropropane nitronate with respect to 2-nitropropane—possible involvement of free radical species. *Mutat. Res.*, **179**, 15–22.
29. Kido,T., Soda,K. and Asada,K. (1978) Properties of 2-nitropropane dioxygenase of *Hansenula mrakii*. *J. Biol. Chem.*, **253**, 226–232.
30. Kido,T. and Soda,K. (1984) Oxidation of anionic nitroalkanes by flavoenzymes, and participation of superoxide anion in the catalysis. *Arch. Biochem. Biophys.*, **234**, 468–475.
31. Mermelstein,R., Kiriazides,D.K., Butler,M., McCoy,E.C. and Rosenkranz,H.S. (1981) The extraordinary mutagenicity of nitropyrenes in bacteria. *Mutat. Res.*, **89**, 187–196.

Received on November 23, 1987; revised on February 10, 1988; accepted on February 17, 1988