Modulation of Repair of O₆-Methylguanine in Parenchymal and Nonparenchymal Liver Cells of Rats Treated with Dimethylnitrosamine

Ghyslaine Planche-Martel, Alexei Likhachev, Christopher P. Wild, and Ruggero Montesano

Unit of Mechanisms of Carcinogenesis, International Agency for Research on Cancer, 150 Cours Albert-Thomas, 69372 Lyon Cedex 08, France

ABSTRACT

Previous studies have shown that chronic treatment of rats with dimethylnitrosamine (DMN) (2 mg/kg for 3 weeks) results in increased repair of O₆-methylguanine (O₆-mGua) in liver DNA. The experiments reported here try to determine if this increased repair is confined to one or more cell populations of the liver. Liver cells [parenchymal (PC) and nonparenchymal (NPC)] were separated by elutriation centrifugation at various times after the last administration of DMN.

The in vivo alkylation studies show that at any time after a dose of [¹⁴C]DMN (2 mg/kg) the level of O₆-mGua in PC cells of DMN pretreated rats was much lower than in the same cell population from control rats receiving only a single dose of DMN. In contrast, the pretreatment schedule resulted in no change in the levels of this DNA adduct in NPC cells.

These results were confirmed by the determination of the levels of O₆-methyldeoxyguanosine by radioimmunoassay in DNA from PC or NPC cells of rats similarly either pretreated for 3 weeks with DMN or receiving a single dose of DMN (2 mg/kg). The in vitro measurements of O₆-mGua DNA alkyltransferase activity, using alkylated DNA as substrate, also show a higher activity of this repair enzyme in PC cells. The DMN pretreatment resulted in a 25-fold difference in O₆-mGua DNA alkyltransferase activity between the two cell populations of the liver.

INTRODUCTION

The study of the mechanisms underlying the specificity of the carcinogenic action of the nitrosamines in a given animal species, organ, or cell type provides one of the most challenging areas of research in experimental carcinogenesis. It both provides a valuable system for assessing the role of the various determinants of the carcinogenic process, and is a source of biochemical knowledge which can be valuable in the extrapolation of carcinogenicity data across various species, including humans. Among the determinants that appear critical in the initiation of carcinogenesis by nitrosamines (1), it is DNA alkylation and subsequent DNA repair processes which seem the most relevant (2–5) and in particular, repair of alkylation damage at the oxygen atoms of purine and/or pyrimidine bases (6, 7).

In rats the inability of a single dose of DMN to induce tumors in the liver (except when the nitrosamine is given after partial hepatectomy) is associated with the high capacity of this organ to repair O₆-mGua from its DNA (8, 9). In contrast, chronic treatment with DMN results in the induction of liver tumors (10), and information from various large scale dose-response carcinogenicity studies carried out with DMN in rats are available (11–13). Repeated administration of DMN to BD IV rats has been shown to increase specifically the repair of O₆-mGua in liver (14), and this effect has now been observed under various experimental conditions (15). Previous studies (14, 16, 17) in our laboratories have shown that the increased repair of O₆-mGua from total liver DNA (a) was dependent, both upon the dose and the length of DMN treatment; (b) persists for various weeks upon cessation of the treatment; and (c) this increased repair capacity was limited to repair of relatively low levels of O₆-mGua; in addition it was shown that increased repair of O₆-mGua does not appear to be linked to increased DNA synthesis. All these studies were carried out using DNA from whole liver, while here we report results obtained for the two liver cell populations, PC and NPC cells, considered individually under conditions which were found to be optimal for the induction of the increased repair of O₆-mGua. Following DMN treatment the disappearance of O₆-mGua from the two cell populations in vivo was measured either radiochemically or by RIA, and in parallel the O₆-mGua DNA alkyltransferase activity of crude protein extracts from PC or NPC cells was determined.

MATERIALS AND METHODS

Chemicals

[¹⁴C]DMN (57.3 to 60.7 mCi/mmol) was synthesized in the Paterson Laboratories, Manchester, United Kingdom, from [¹⁴C]dimethylamine hydrochloride. Unlabeled DMN was obtained from Merck-Schuchardt, Munich, Federal Republic of Germany. Collagenase type IV, obtained from the Sigma Chemical Co., St. Louis, MO, and collagenase, obtained from Boehringer Mannheim, France, were always tested for activity before use, due to marked batch variations. All the other compounds used were of the purest grade available.

Experimental Design

The animals used in all experiments were male BD IV rats bred in the animal house of the International Agency for Research on Cancer. The animals received food and water ad libitum, except that they were fasted overnight before perfusions.

Experiment 1. Rats weighing 110–150 g at the start of the experiment received a daily dose on weekdays of 2 mg of unlabeled DMN/kg by stomach intubation for 3 weeks. The last dose on the 14th or 15th treatment day of the experiment was 2 mg [¹⁴C]DMN/kg i.p. 24 h after the last administration of unlabeled DMN to the above rats. The same dose of [¹⁴C]DMN (2 mg/kg i.p.) was administered to a group of control rats not pretreated with unlabeled DMN. Liver perfusions and separations...
of parenchymal and nonparenchymal cells by elutriation centrifugation (see below) were performed 2, 6, or 10 h after administration of the radioactive carcinogen. The cells were frozen in liquid N₂ and stored at −80°C prior to DNA extraction. Results for each time point were obtained from 2 or 3 rats and the variation observed was less than 20%.

Experiment 2. Rats were pretreated with unlabeled DMN (2 mg/kg) for 3 weeks as in Experiment 1, and 2, 6, or 24 h after the last administration of unlabeled DMN the livers were perfused and the PC and NPC cells were separated. Another group of rats received a single dose of unlabeled DMN by stomach intubation, and 2, 6, or 24 h later the two types of liver cells were isolated. Cells were frozen and stored at −80°C for subsequent analysis of O₆-mdGuo content in DNA by RIA, using a monoclonal antibody specific for this alkylated base. These antibodies were kindly provided by Drs. Boyle and Saffhill (Paterson Laboratories, Christie Hospital, and Holt Radium Institute, Manchester, United Kingdom) (18). For each time point the results were obtained from 1–2 rats.

Experiment 3. Rats were pretreated with unlabeled DMN (2 mg/kg) for 3 weeks by stomach intubation as described above, and 2, 6, or 24 h after the last administration of unlabeled DMN the livers were perfused and the PC and NPC cells were separated. Control samples were obtained from rats which received no treatment. Cells were frozen and stored as above. Cell extracts were subsequently prepared (see below) for determination of O₆-mdGuo DNA alkyltransferase activity.

Liver Cell Separation

All rats, received an i.p. injection of 1250 IU heparin and were anesthetized with sodium phenobarbital prior to perfusion. Livers were perfused in situ by a collagenase technique adopted from Seglen (19), and the resulting mixed liver cell suspension was separated into PC and NPC cells by elutriation centrifugation, using a modification of the method described by Lewis and Swenben (20).

Cell debris and blood were removed from the NPC fraction by using metrizamide gradients and two purified populations were obtained. Cell yields per liver varied from 40 to 180 x 10⁸ NPCs (which include endothelial and Kupffer cells), and from 300 to 700 x 10⁸ PCs with 96 to 98% and 80 to 85% viability, respectively. No difference was observed in cell yield from liver of control or pretreated rats. The whole separation procedure took approximately 6 h, after which cells were frozen in liquid N₂ and stored at −80°C until use.

In Experiments 1 and 2 DNA was isolated from PC and NPC cells according to the procedure described by Umbenhauer and Pegg (21), with some modifications. At the end of the extraction from the PC pellet, DNA was redissolved in water and the solution was ultra centrifuged at 30,000 rpm for 1 h to remove glycerogen. In the case of NPC cells, only one extraction with chloroform:isoamycolol (10:1; v/v) was used and there was no incubation with Pronase. DNA was stored at −30°C until analysis. The yield of DNA was approximately 14 μg/10⁶ cells for PC and 8 μg/10⁶ cells for NPC.

In Experiment 1 the DNA was hydrolyzed to release free purine bases by heating at 70°C for 30 min in 0.1 N HCl. After addition of unlabeled markers of methylated purines (3-mAde, 7-mdGuo, and O₆-mdGuo) the sample was adjusted to pH 2.8-2.9, and then chromatographed on a column of Sephadex G-10 (90 x 0.6 cm) and eluted with 0.05 M ammonium formate containing 0.02% (w/v) sodium azide, pH 6.7.5. The amounts of guanine and adenine in the sample were calculated from the UV absorption of the fractions containing these bases. The amounts of methylated bases present are expressed as mol/10⁶ mol of the parent base. Further details of this analytical procedure are given elsewhere (22).

The measurements by RIA in Experiment 2 were performed using the system and the monoclonal antibodies described by Wild et al. (18). DNA extracted from PC and NPC cells was dissolved in buffer (10 mM Tris buffer:5 mM MgCl₂:1 μM 2-deoxycoformycin (National Cancer Institute, Bethesda, MD)) for enzymic hydrolysis. Digestion was carried out with 200 units DNase I/ml (Boehringer Mannheim) at 37°C for 2 h, pH 7.1, followed by adjustment to pH 8 with 0.5 M Tris buffer. Digestion was completed by adding alkaline phosphatase, 170 units/ml (Boehringer) and snake venom phosphodiesterase, 0.075 units/ml (Boehringer Mannheim) and incubating overnight at 37°C. Enzymes were removed by ethanol precipitation, and hydrolysates were air dried, reconstituted in 0.8 ml 10 mM ammonium bicarbonate, and applied to an Aminex A7 cation exchange separation system consisting of two columns (5 x 0.75 cm) and (30 x 0.75 cm) in series. Separations were carried out at 50°C using 10 mM ammonium bicarbonate, pH 8, as eluting buffer at a flow rate of 0.75 ml/min. The eluate was monitored at 254 nm and fractions containing individual parent deoxynucleosides were quantitated spectrophotometrically. Fractions containing O₆-mdGuo were pooled, air dried, and reconstituted in 350 or 400 μl of buffer (phosphate-buffered saline; 1 μM deoxycoformycin; 1% fetal calf serum; 3 M sodium azide) for RIA.

**Assay for O₆-Methylguanine DNA Alkyltransferase**

Cells were homogenized by vortexing in 1–2 ml of buffer (50 mM Tris:0.1 mM EDTA:1.0 mM dithiothreitol, pH 7.5) and sonicated three times at 4°C for 30 s at 1-min intervals. The mixture was centrifuged at 12,000 x g for 10 min, the pellet was resuspended in 1 ml of the same buffer, sonicated, and centrifuged as above. The combined supernatants were centrifuged again at 12,000 x g for 30 min, and the protein concentration was determined by the Bio-Rad protein assay (23, 24). All the extracts were stored at −80°C until use.

The O₆-mdGuo DNA alkyltransferase assay was carried out as described by Hall et al. (25). The assay mixture, total volume 3 ml, always contained 0.15 mg [methyl-³H]DNA (with 364 femtol mole of O₆-mdGuo; 1300 dpm) but different amounts of protein (0.5 mg up to 20 mg). After incubation (37°C for 1 h) the reaction was stopped by the addition of 0.1 volume of saturated sodium acetate and 2 volumes of ice-cold ethanol.

The DNA was hydrolyzed in 0.1 M perchloric acid for 2 h, and the purine bases were separated, using a Sephadex G-10 column or a Partisil 10/25 SCX column when high-pressure liquid chromatography separation was used. In each set of experiments a control was included in which the cell extract was incubated separately from the substrate DNA and mixed with it at the time of precipitation. For each sample the removal capacity was calculated from the total dpm present as O₆-mdGuo in the control, and at the same time it was checked that no variations had occurred at the level of 7-mdGuo. Three to four rats were used individually for each time point. The results are expressed as mean values of these assays.

**Carcinogenicity Experiments**

Groups of 29 male BD IV rats (weighing 90–120 g at the start of the experiment) were treated with a daily dose (2 mg/kg) of DMN by stomach intubation for 24 weeks (5 days/week) and were observed up to 120 weeks of age. Groups of 29 control rats received saline and were followed for the same period of time.

**RESULTS**

**In Vivo Alkylation in Parenchymal and Nonparenchymal Liver Cells.** Table 1 shows the levels of 3-mAde, O₆-mdGuo, and 7-mdGuo in DNA of PC and NPC liver cells at various times after a dose of [¹³C]DMN (2 mg/kg) to control rats, or to rats that had previously received unlabeled DMN at a dose of 2 mg/kg on weekdays for a period of 3 weeks. In both cell types the levels of 3-mAde at all three time points were very similar in control and pretreated rats. Also, no significant differences were observed in the 7-mdGuo levels, although a slightly higher level was detected at 2 h in both cell types of the pretreated rats as
were in some instances very small. As shown in Chart 1 the O6-

compared to controls; this effect was not evident at 6 or 10 h.

In addition, there was a small effect in NPC cells where a lower level of 7-mGua was observed compared to PC cells in both control and pretreated rats at 2, 6, and 10 h. However, in the case of O6-mGua, marked differences were observed between the two cell populations, in that the level of this adduct in the DNA of PC cells from pretreated rats 2 h after the dose of [14C] DMN was less than one-half (16 mol/108 mol of guanine) the level observed in PC cells from control rats (37 mol/108 mol of guanine); even greater differences were detected at 6 and 10 h. In contrast, in the DNA of NPC cells no such difference in O6-
mGua level between pretreated and control rats was observed and in fact, at 2 h a higher level was observed in pretreated versus control rats (Table 1).

The differences in the relative amounts and the persistence of O6-mGua in the two types of liver cells from pretreated or control rats are also evident in Chart 1, showing the O6-mGua:7-mGua ratios at 2, 6, and 10 h. These ratios, based on the radioactivity present in different fractions from the same column eluate, are probably a more accurate representation of the observed differences, since the individual measurement of alkylated bases depends on the determination of the actual amount of the parent base present in DNA, and the quantities obtained from NPC cells were in some instances very small. As shown in Chart 1 the O6-
mGua:7-mGua ratios in PC cellular DNA from pretreated rats were one-third the corresponding ratios in control rats at 2, 6, and 10 h; in DNA from NPC cells no significant differences are evident between the two groups of rats. In this Chart the O6-
mGua:7-mGua ratios observed previously (16) in total liver DNA in groups of rats similarly treated are shown for comparison. It is evident that the specific increased repair of O6-mGua in pretreated rats is confined to the PC cell population of the liver. The specificity of this effect for the O6-mGua lesion is further indicated by the fact that the other two DNA alkylation products measured (3-mAde and 7-mGua) are not affected by the DMN pretreatment (see Table 1).

In Experiment 2 the levels of O6-mGua were determined by RIA in DNA from PC or NPC liver cells of rats pretreated for 3 weeks with DMN or receiving a single dose of DMN (2 mg/kg). The RIA was used as it allowed a greater sensitivity than radioactive determinations, and this proved especially important for measurements with NPC cells where, as mentioned above, much less DNA was available for analysis. In agreement with the studies using [14C]DMN (Table 1), the results from the RIA analysis (Table 2) also showed that the levels of O6-mGua in PC cells from rats receiving pretreatment were much lower, at 2 and 6 h posttreatment, than those seen in the same cell population from rats receiving only a single dose of DMN. Even 24 h after the final dose of DMN, a time point not examined in Experiment 1, the level of O6-mGua in the pretreated group was one-half that found in the controls. The O6-mGua measurement for NPC cells, from both pretreated and control rats, again showed no differences between the two groups at 2 and 6 h, emphasizing that the discrepancy between pretreated versus control rats is limited to the PC cell population only. There was some evidence of lower O6-mGua levels in NPC cells from pretreated rats at 24 h when compared to controls (see also Table 1), but this small difference requires further investigation to determine whether it is a real difference between the two groups.

Table 2

<table>
<thead>
<tr>
<th>Time (hr) after [14C] DMN administration</th>
<th>Parenchymal cells</th>
<th>Nonparenchymal cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretreated</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>68</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>24</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>
with DMN (2 mg/kg), and is in accord with the increased cell replication which was observed in NPC cells of F344 rats measured by [3H]thymidine and bromodeoxyuridine labeling following chronic administration of 1,2-dimethylhydrazine (26).

**O**<sup>6</sup>-**Methylguanine DNA Alkyltransferase Activity in Parenchymal and Nonparenchymal Cells.** The results obtained in PC and NPC liver cells of the O<sup>6</sup>-mGua DNA alkyltransferase activities are directly complementary to the in vivo findings. Chart 2 shows that in control rats a 50% repair of O<sup>6</sup>-mGua from the DNA (containing 364 fmol O<sup>6</sup>-mGua/150 μg DNA) occurs with 4 × 10<sup>6</sup> PC cells, whereas 13.5 × 10<sup>6</sup> NPC cells were required for similar removal. In the rats pretreated with 2 mg DMN/kg for 3 weeks in PC liver cells assayed 24 h after the last DMN injection, 50% repair occurs with 1 × 10<sup>6</sup> cells; in NPC cells no increased activity was observed following DMN treatment, and if anything a lower activity was detected versus the corresponding NPC control cells. The results are expressed per viable cell (and not per mg protein), as this is a more correct representation of the results, since the amount of protein per cell greatly differs between the two cell populations analyzed (24, 27). The difference in the O<sup>6</sup>-mGua DNA alkyltransferase between PC and NPC cells in control rats is very similar to that observed in another strain of rats (24). The DMN pretreatment resulted in a 25-fold difference in O<sup>6</sup>-mGua DNA alkyltransferase activity between the two cell populations of the liver (Table 4).

**Carcinogenicity Studies.** Chronic administration of daily doses of 2 mg of DMN/kg for a total of 24 weeks resulted in 25 tumor-bearing animals, of which 24 developed tumors of liver; these were 19 hepatocellular carcinomas and 7 hemangioendothelial tumors. One rat had a glioma and a mesenchymal tumor. No liver tumors were observed. After cessation of continuous exposure to DMN (2 mg/kg for 3 weeks), control rats received [14C]DMN only, while rats previously treated with 2 mg DMN/kg for 3 weeks in PC liver cells assayed 24 h after the last DMN injection, 50% repair occurs with 1 × 10<sup>6</sup> cells; in NPC cells no increased activity was observed following DMN treatment, and if anything a lower activity was detected versus the corresponding NPC control cells. The results are expressed per viable cell (and not per mg protein), as this is a more correct representation of the results, since the amount of protein per cell greatly differs between the two cell populations analyzed (24, 27). The difference in the O<sup>6</sup>-mGua DNA alkyltransferase between PC and NPC cells in control rats is very similar to that observed in another strain of rats (24). The DMN pretreatment resulted in a 25-fold difference in O<sup>6</sup>-mGua DNA alkyltransferase activity between the two cell populations of the liver (Table 4).

**DISCUSSION**

The aim of the present and previous studies (14, 16, 17) was to examine the modulation of DNA repair processes dealing with various DNA alkylation adducts during chronic administration of nitrosamines.

The initial level (2 h) of 7-mGua was slightly higher in PC compared to NPC cells (Table 1). In order to alkylate DNA, it is proposed (28) that DMN has to be metabolized through an oxidative demethylation to the unstable nitrosohydroxymethylmethylamine which generates a methyl carbonium ion. It is thus probable that in vivo the metabolic activation of DMN occurs in liver PC cells, and the methylation species crosses the cellular membrane and reacts with DNA of nonhepatic cells of the liver, which are limited in or lack completely this metabolic competence. This is consistent with the finding of Umbenhauer and Pegg (29), showing that freshly isolated hepatocytes are able to metabolize DMN, and that the intermediate nitrosohydroxymethylmethylamine, with an estimated half-life of 10 s, (30), is able to alkylate extracellular DNA. Thus although some differences in initial alkylation are observed (Table 1) (31), the difference in susceptibility of the various cell populations of the liver should be considered in terms of two events occurring subsequent to carcinogen interaction with cellular DNA repair and DNA synthesis. In pretreated rats, both NPC and PC cells show the highest 7-mGua levels 2 h after exposure as compared to 6 h in control rats. The data suggest that chronic exposure to DMN causes some induction of metabolizing enzymes as well as modulating repair, as discussed below.

Treatment of rats with 2 mg DMN/kg for a period of 3 weeks results in an increased repair of O<sup>6</sup>-mGua, but not of 7-mGua or 3-mAde, in DNA obtained from the liver as a whole (16), and this effect persists for several weeks after cessation of the DMN treatment (17). This specific increase of O<sup>6</sup>-mGua repair in liver DNA is confined to the PC cells. In fact Chart 1 shows that the difference between pretreated and control rats in the O<sup>6</sup>-mGua:7-mGua ratios in total DNA or in the DNA from PC cells is of the same order of magnitude (approximately 4- to 5-fold). The O<sup>6</sup>-mGua DNA alkyltransferase activity also confirms (see Chart 2) that in control rats PC cells have approximately four times higher activity than in NPC cells, and this difference increases to 25-fold following pretreatment with DMN. Calculation of the number of molecules of O<sup>6</sup>-mGua DNA alkyltransferase per cell (55,000 for PC and 7,800 in NPC liver of control rats and 110,200 and 4,500 in the DMN-treated rats) are very close to those reported in the literature (5). Similarly, in BD IX and F344 rats (20, 24, 31) chronic treatment with 1,2-dimethylhydrazine resulted in an increased repair of O<sup>6</sup>-mGua only in the PC cells, and the magnitude of the increased repair was of the order observed in our...
of any possible difference in carcinogenic susceptibility between PC and NPC cells. It should be stressed in all cases that these considerations be taken in the context of the stochastic process of carcinogenesis, and that the contrasting carcinogenic response of the different liver cell populations is not so clear-cut and is greatly affected by the dose of carcinogen. For example a recently reported (13) large scale study in Colworth rats shows that the induction by DMN of hepatocellular carcinomas is approximately linear in the dose range below 0.1 mg/kg/day, and an approximately cubic relationship is observed within the higher range of doses. This “hockey stick” carcinogenic dose response could be possibly related to the findings reported here and previously (14, 16, 17) of an increased repair of O6-mGua in DNA of hepatocytes after chronic treatment with relatively low doses of DMN, and in the saturation of this DNA repair process when high doses are used (33, 34). It is also evident from the experiments in Colworth rats that this repair process, although efficient and assuming that it is directly related to the frequency of cancer induction by nitrosamines, cannot reduce to zero the probability of developing tumors. In Escherichia coli the induction of O6-mGua DNA alkyltransferase is of a much higher level, going from 20 to 10,000 molecules/cell, and resulting in a more drastic reduction of mutation frequency after a challenging dose of N-methyl-N'-nitro-N-nitrosoguanidine (35, 36).

Swenberg et al. (37) observed in rats an accumulation of O4-ethyldeoxythymidine but not of O6-ethyldeoxyguanosine in DNA of hepatocytes following treatment with diethylnitrosamine, which induces a high incidence of hepatocellular carcinomas. This result points to O4-ethyldeoxythymidine as a more critical DNA lesion in this instance, and suggests that these two ethylation DNA adducts may be repaired by different enzymes or, if indeed the same DNA alkyltransferase is involved, then it has a much higher frequency of removal of O4-ethyldeoxyguanosine than of O4-ethyldeoxythymidine. In E. coli the purified O6-mGua DNA alkyltransferase repairs both O6-mGua and O4-methyldeoxythymidine (38), and in mammalian cells there is also strong evidence that the same DNA alkyltransferase acts on both lesions although with a different affinity (39). This is an important point that requires clarification and it should also be noted that other O-alkylated pyrimidines may be involved in initiation, but studies are as yet limited by lack of sensitive assay methods. Specificity at a cell population level in relation to initial DNA damage and subsequent DNA repair processes is certainly relevant to nitrosamine carcinogenesis; however an aspect that has received so far little attention is the possible specificity of distribution of these DNA adducts within chromatin structure of the cellular genome. Recently Nehls et al. (40) have shown the occurrence of highly sensitive sites for O6-ethylguanine adducts in brain DNA following in vivo treatment of rats with ethylnitrosourea. This observation is of particular interest since it was observed (41, 42) that in mammary tumors induced in rats by N-methyl-N-nitrosourea the activation of H-ras oncogene was the result of a single point mutation (G→A transition), which is precisely what one would expect from a defective repair of O6-mGua. Both these observations imply the occurrence of “hot spots” of alkylolation within the DNA which are of relevance to the carcinogenesis process.

ACKNOWLEDGMENTS

We would like to thank O. Deblock for her technical help, Dr. R. Becker for his criticisms, Dr. J. R. P. Cabral for his consultation on the pathology, and P. Collerd-Bianchi for her secretarial help.

### Table 4

**O6-Methylguanine DNA alkyltransferase activity in parenchymal and nonparenchymal cells of control rats and rats treated with DMN**

<table>
<thead>
<tr>
<th>Activity in PC</th>
<th>Activity in NPC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fmol/h/mg protein</td>
</tr>
<tr>
<td>Control</td>
<td>71 ± 23</td>
</tr>
<tr>
<td>Treated</td>
<td>148 ± 42</td>
</tr>
</tbody>
</table>

The prevalence of liver tumors originating from NPC cells has been associated with the low capacity of these target cells to repair O6-mGua and the concomitant high level of DNA synthesis induced by the carcinogen treatment (24, 31). The dose of DMN and the duration of treatment in the carcinogenicity experiments reported here however, in which mainly hepatocellular carcinomas were induced, were too high to allow detection of any possible difference in carcinogenic susceptibility between PC and NPC cells.

In the experiments quantitated by RIA we are measuring total O6-mdGuo, including both alkylation from pretreatment schedules as well as from the final dose of DMN. One might expect to find therefore an accumulation of this lesion in NPC cells upon chronic exposure, as the O6-mGua repair capacity is apparently not increased sufficiently to remove all of the lesion in 24 h. The results reported here however show no difference of O6-mdGuo levels in pretreated NPC cells compared to controls 2 h after the final dose of DMN (Table 2). This suggests that any O6-mdGuo present due to accumulation is small in comparison to the quantity of this adduct induced by such a dose of this carcinogen. This observation is in accord with that of Bedell et al. (31), where accumulation of O6-mdGuo/mg DNA in NPC cells was seen up to 8 days during daily exposure to 1,2-dimethylhydrazine, but that the concentration of the adduct in DNA declined markedly over the next 3 weeks. It has been reported that the rate of de novo DNA synthesis in NPC cells is higher than in PC cells in control rats, and also is increased far more in the former cell type compared to the latter in response to daily exposure to 1,2-dimethylhydrazine (26). We measured metabolic incorporation of [14C]DMN into adenine via the C-1 pool and found 20-fold and 40-fold more incorporation in control and pretreated NPC cells compared to PC control and pretreated cells, respectively (Table 3). Thus a large increase in DNA synthesis specifically in NPC cells would lead to a dilution of the concentration of O6-mdGuo in this cell population, resulting in an apparent reduction of accumulation. Under these circumstances it might be argued that it is difficult to eliminate the possibility that part of the lack of O6-mdGuo accumulation in NPC cells is a reflection of a small induction of O6-mGua DNA alkyltransferase activity masked by the “dilution” effect discussed above. The situation would be made clearer from a knowledge of the level of 7-mdgua or 3-mdAde, which were not determined in this instance. However the data from the alkyltransferase studies (Chart 2) show no evidence of increased repair in NPC cells and again strongly suggest that such an increase is limited to the PC cell population.

In rats treated with 1,2-dimethylhydrazine and in mice after DMN, liver hemangiendothelial tumors are more frequently observed, hepatocellular tumors however are also reported (31, 32). The prevalence of liver tumors originating from NPC cells has been associated with the low capacity of these target cells to repair O6-mGua and the concomitant high level of DNA synthesis induced by the carcinogen treatment (24, 31). The dose of DMN and the duration of treatment in the carcinogenicity experiments reported here however, in which mainly hepatocellular carcinomas were induced, were too high to allow detection of any possible difference in carcinogenic susceptibility between PC and NPC cells. It should be stressed in all cases that these considerations be taken in the context of the stochastic process of carcinogenesis, and that the contrasting carcinogenic response of the different liver cell populations is not so clear-cut and is greatly affected by the dose of carcinogen. For example a recently reported (13) large scale study in Colworth rats shows that the induction by DMN of hepatocellular carcinomas is approximately linear in the dose range below 0.1 mg/kg/day, and an approximately cubic relationship is observed within the higher range of doses. This “hockey stick” carcinogenic dose response could be possibly related to the findings reported here and previously (14, 16, 17) of an increased repair of O6-mGua in DNA of hepatocytes after chronic treatment with relatively low doses of DMN, and in the saturation of this DNA repair process when high doses are used (33, 34). It is also evident from the experiments in Colworth rats that this repair process, although efficient and assuming that it is directly related to the frequency of cancer induction by nitrosamines, cannot reduce to zero the probability of developing tumors. In Escherichia coli the induction of O6-mGua DNA alkyltransferase is of a much higher level, going from 20 to 10,000 molecules/cell, and resulting in a more drastic reduction of mutation frequency after a challenging dose of N-methyl-N'-nitro-N-nitrosoguanidine (35, 36).
REFERENCES


7. Singer, B. Alkylation of the O-b of guanine is only one of many chemical events which may initiate carcinogenesis. Cancer Invest., in press, 1985.


Modulation of Repair of $O^6$-Methylguanine in Parenchymal and Nonparenchymal Liver Cells of Rats Treated with Dimethylnitrosamine

Ghyslaine Planche-Martel, Alexei Likhachev, Christopher P. Wild, et al.