Nucleotide excision repair modulates the cytotoxic and mutagenic effects of N-n-butyl-N-nitrosourea in cultured mammalian cells as well as in mouse splenocytes in vivo


1 MGC–Department of Radiation Genetics and Chemical Mutagenesis, Leiden University, PO Box 9503, 2300 RA Leiden, 2 National Institute of Public Health and the Environment, Department of Carcinogenesis, Mutagenesis and Genetics, Bilthoven and 3 J.A. Cohen Institute, Interuniversity Research Institute for Radiopathology and Radiation Protection, Leiden, The Netherlands

The butylating agent N-n-butyl-N-nitrosourea (BNU) was employed to study the role of nucleotide excision repair (NER) in protecting mammalian cells against the genotoxic effects of monofunctional alkylating agents. The direct acting BNU was found to be mutagenic in normal and XPA mouse splenocytes after a single i.p. treatment in vivo. After 25 and 35 mg/kg BNU, but not after 75 mg/kg, 2- to 3-fold more hprt mutants were detected in splenocytes from XPA mice than from normal mice. Using O6-alkylguanine-DNA alkyltransferase (AGT)-deficient hamster cells, it was found that NER-deficient CHO UV5 cells carrying a mutation in the ERCC-2 gene were 40% more mutable towards lesions induced by BNU when compared with parental NER-proficient CHO AA8 cells. UV5 cells were 1.4-fold more sensitive to the cytotoxic effects of BNU compared with AA8 cells. To investigate whether this increased sensitivity of NER-deficient cells is modulated by AGT activity, cell survival studies were performed in human and mouse primary fibroblasts as well. BNU was 2.7-fold more toxic for mouse XPA fibroblasts compared with normal mouse fibroblasts. Comparable results were found for human fibroblasts. Taken together these data indicate that the role of NER in protecting rodent cells against the mutagenic and cytotoxic effects of the alkylating agent BNU depends on AGT.

Introduction

Nucleotide excision repair (NER) is an evolutionarily conserved DNA repair pathway which protects cells against the genotoxic effects of a wide range of DNA damaging agents. NER recognizes and removes DNA lesions that induce structural distortions in the DNA helix. At present, it is well established that in cultured mammalian cells NER efficiently recognizes and removes UV-induced photoproducts as well as bulky DNA lesions induced by chemicals such as 2-acetylaminofluorene (Regan and Setlow, 1974; Amacher and Lieberman, 1977; Francis et al., 1981), benzo[al]pyrene (Maher et al., 1976) and aflatoxin B1 (Leadon et al., 1981; Thompson et al., 1983; Martin and Waters, 1992). Recently, it has been shown that the NER protein XPG is involved in the removal of thymine glycols in human cells (Cooper et al., 1997). In addition to bulky lesions, it has been shown that NER removes abasic sites and thymine glycols in cell-free systems (Lin and Sancar, 1989; Snowden et al., 1990; Huang et al., 1994; Hess et al., 1997).

The broad substrate specificity of NER raises the question as to whether this DNA repair pathway removes DNA lesions induced by monofunctional alkylating agents (MAA) as well. MAA are a group of chemical agents that react with oxygen and nitrogen atoms in the DNA and which are well characterized with respect to genotoxicity and carcinogenicity (Druckrey et al., 1967, Singer and Grunberger, 1983). In Escherichia coli, O6-methylguanine, O6-ethylguanine and O4-ethylthymine are removed by NER in strains expressing no or low levels of Ada, one of the E.coli O6-alkylguanine-DNA alkyltransferases (AGT) which remove predominantly alkyl groups from the O6 position of guanine (Samson et al., 1988; Friedberg et al., 1995). In addition, a positive correlation is found in E.coli between the size of the alkyl group and the sensitivity for killing and mutation induction when strains deficient in NER are exposed to series of MAA (Garner et al., 1979; Yoshikawa et al., 1980; Todd and Schendel, 1983; Kohda et al., 1987). In mammalian cells, however, the role of NER in protecting cells against alkylolation-induced cytotoxicity and mutagenicity is still unclear. Mammalian NER is probably not involved in the repair of O6-methylguanine, since this lesion persists in NER-proficient hamster cells deficient for AGT (Bignami et al., 1989), although O6-methylguanine has been shown to be a substrate for NER in a human cell-free system (Huang et al., 1994). Ethylated DNA damage is shown to be a substrate for NER in mammalian cells, although it remains to be determined which DNA adducts are removed by NER and whether AGT cooperates with NER (Maher et al., 1990; Bronstein et al., 1991, 1992; Op het Veld et al., 1997).

In the present study the effect of NER on cytotoxicity and mutagenicity induced by MAA in mammalian cells was investigated using N-n-butyl-N-nitrosourea (BNU). BNU is a direct acting butylating agent that induces upon reaction with oxygen and nitrogen atoms of the bases in DNA O6-n-butylguanine (8.4%), O2-n-butylthymine (8.2%), O4-n-butylthymine (0.8%), 3-n-butylthymine (0.1%), 3-n-butyladenine (13.2%) and 7-n-butylguanine (12.8%), although the complete DNA adduct spectrum is unknown (Saffhill, 1984). In addition to the predominant n-butyl lesions, BNU also induces a small proportion of rearranged secondary butyl adducts which are formed after prolonged exposure (Saffhill, 1984; Saffhill et al., 1985). O6-n-butylguanine is a mutagenic lesion (Chambers et al., 1988; Chambers, 1991; Baumgart et al., 1993) that is a substrate for AGT in the rat under in vitro conditions (Morimoto et al., 1985). O6-n-butylguanine is probably not recognized by human AGT (Liem et al., 1994) and AGT-independent removal of O6-n-butylguanine has been found in hamster and human cells (Boyle et al., 1986a,b). Furthermore, O6-n-butylguanine is poorly removed in NER-deficient xeroderma pigmentosum.

To whom correspondence should be addressed. Tel: +31 71 5276126; Fax: +31 71 5221615; Email: mullenders@rull2.medfac.leidenuniv.nl

© UK Environmental Mutagen Society/Oxford University Press 1999
group A (XP-A) human fibroblasts that are proficient or deficient for AGT, indicating that NER is involved in the removal of O\(^2\)-methylguanine (Boyle et al., 1986b).

To assess the biological relevance of NER in the removal of BNU-induced lesions under in vivo conditions, the knock-out mouse model lacking a functional XPA gene product was used (De Vries et al., 1995). The XPA mouse has been shown to be a suitable in vivo model to study NER, since XPA mice, like XP-A patients, are highly susceptible to the induction of skin tumours by UVB (De Vries et al., 1995). Moreover, internal lymphoid tissue in XPA mice is also affected by the NER deficiency as demonstrated by studying benzo[a]pyrene-induced mutagenesis (De Vries et al., 1997; Bol et al., 1998). XPA mice and normal littermates were exposed to BNU and subsequently mutation induction was studied at the hprt gene in T lymphocytes isolated from the spleen. The in vivo analyses were complemented by studies on the effect of NER on BNU-induced mutagenesis at the Hprt locus in the AGT-deficient CHO cell lines AA8 and its NER-deficient derivative UV5. The latter carries a mutation in the ERCC-2 gene, the hamster homologue of the human XPD gene (Weber et al., 1994). The protective role of NER on BNU-induced cytotoxicity was studied in the CHO cell lines AA8 and UV5 as well as in primary mouse and human XP-A fibroblasts.

Materials and methods

**Synthesis of BNU**

BNU was synthesized from n-butylurea (Aldrich, St Louis, MO) according to Vogel (1957). The crystalized product consists of >95% BNU as determined by reverse phase high performance liquid chromatography and UV detection at 224 nm. Purified BNU was stored at -20°C. BNU was dissolved in 80% dimethylsulphoxide (DMSO)/water and kept at room temperature for a maximum of 10 min until use.

**Cell culture of Chinese hamster ovary cells and primary fibroblasts**

The CHO cell line UV5 and its parental line AA8 were cultured in medium consisting of HAM’s F-10 medium without hypoxanthine and thymidine supplemented with 15% newborn calf serum and antibiotics (100 U/ml penicillin and 0.1 mg/ml streptomycin). Cells were cultured at 37°C in a 5% CO\(_2\) atmosphere.

Human wild-type (VH25D) and XP-A (XP25RO) fibroblasts, as well as mouse embryonic fibroblasts (MEFs), were cultured in Dulbecco’s modified Eagle’s medium (DMEM; 2 mM glutamine, and 3.7 g/l NaHCO\(_3\)) supplemented with 1% glutamine, 1% non-essential amino acids, 10% fetal calf serum (FCS) and antibiotics at 37°C in 2.5% CO\(_2\).

**BNU treatment of hamster cells**

Chinese hamster ovary cells were exposed as monolayers to BNU in 5 ml HAM’s F-10 medium supplemented with HEPES (pH 7.4) containing 100 μl of a BNU stock solution (50X). BNU treatment was performed for 1 h at 37°C in 5% CO\(_2\). The cells were exposed to BNU with a final concentration of up to 12 mM BNU.

**Clonal cell survival and Hprt mutation induction in Chinese hamster ovary cells**

To determine cell survival, cells were seeded in 10 94 mm dishes at a density of 300 (0, 2 and 4 mM BNU) or 600 cells/dish (8 and 12 mM BNU) on the day of treatment. Cells were incubated for 4 h at 37°C in 5% CO\(_2\) to allow attachment to the dish. After BNU treatment, cells were washed twice with complete culture medium and cultured for 10 days. Then the clones were fixed, stained with methylene blue and counted. The relative survival for each exposure level was calculated taking the survival of untreated cell populations as 100%.

To determine the hprt mutant frequency, 94 mm dishes/dose were each seeded with 0.5×10\(^6\) cells, 16 h prior to BNU treatment. One and four days after BNU treatment, cells were trypsinized and reseeded in three 145 mm dishes/dose and 7×10\(^4\) cells/dish for the lower BNU doses (0.2 and 4 mM BNU) and 7×10\(^5\) cells/dish for the higher doses (8 and 12 mM BNU). At 7 days after BNU treatment, 1×10\(^4\) cells/dish were seeded in 10 94 mm dishes/dose and hprt mutants were selected by the addition of 5 μg/ml 6-thioguanine (6-TG) to the culture medium. In parallel, five 94 mm dishes/dose, each containing 200 cells, were used to determine the cloning efficiency (CE) of the cells. The cells were cultured for 10 days, the colonies were fixed, stained with methylene blue and counted. The hprt mutant frequency was calculated from the frequency of 6-TG-resistant clones corrected for the CE.

**Cell survival of mouse and human primary fibroblasts**

Exponentially growing primary mouse and human fibroblasts in 24 well plates were treated with BNU (up to 32 mM) for 1 h in HEPES buffered DMEM (pH 7.4), washed twice with medium without serum and cultured for 5 days. The surviving cells were visualized by the addition of the tetrazolium salt XTT, which is cleaved by mitochondrial enzymes of viable cells into an orange formazan dye (Cell Proliferation Kit II; Boehringer Mannheim). The absorbance was read after 2 h by an enzyme-linked immunosorbent assay reader. The survival of treated cells was calculated as the percentage of absorbance relative to the absorbance of untreated cells.

**Animals and treatment**

At the Institute of Public Health (Bilthoven, The Netherlands) normal (XP-A) and XP-A mice in a C57BL6 genetic background (De Vries et al., 1995) were kept in macrolon cages. Water and food was supplied ad libitum. At the age of 8 weeks, male mice were treated i.p. with a single dose of 0, 10, 25, 35 or 75 mg/kg BNU dissolved in 80% DMSO, in a volume of 5 ml/kg body wt. The mice were killed 7 weeks after treatment and the spleen was isolated.

**Determination of hprt mutant frequencies in mouse splenocytes**

Cell culture. The T lymphocyte culture medium used for priming and cloning of T cells was prepared as described previously (Bol et al., 1998). The SP/0 lymphoblastoid cell line was cultured in SP/GR medium (standard medium (RPFl 1640, 25 mM HEPES, 2 μg/l NaHCO\(_3\) and 2 mM glutamine) supplemented with 10% FCS (Life Technologies, Rockville, MD), 50 μM 2-mercaptoethanol and antibiotics (100 μg/ml penicillin, 100 μg/ml streptomycin)) at 37°C in 5% CO\(_2\) (Bol et al., 1998).

Isolation and priming of T lymphocytes. Mouse T lymphocytes were isolated from the spleen according to Tates et al. (1994). Until use, the cells were frozen in standard medium supplemented with 10% DMSO and 40% FCS using a CryoMed freezing apparatus (Forma Scientific, Marietta, OH) and stored in liquid nitrogen. The T lymphocytes were thawed quickly at 37°C, stored on ice and 10 ml of standard medium/40% FCS was slowly added over 30 min. The splenocytes were stimulated in T lymphocyte culture medium (Tates et al., 1994) supplemented with 4 μg/ml concanavalin A (con A; Pharmacia, Buckinghamshire, UK), for ~44 h at 37°C in 5% CO\(_2\).

Selection for hprt mutants. The selection for hprt mutants was done according to Tates et al. (1994). Essentially, stimulated cells were plated in round bottomed 96-well microtiter plates (Nunc, Rochester, NY), at a cell density of 2×10^5 cells/well (672 wells in total) together with 5×10^3 SP2/0 feeder cells/well (SP2/0 cells irradiated with 30 Gy X-rays). The mutants were selected in T lymphocyte culture medium supplemented with con A (1 μg/ml) and 6-TG (2.5 μg/ml). To determine the clone forming ability, stimulated cells were counted and plated at a cell density of 6 cells/well (in total 288 wells) in the presence of 5×10^3 SP2/0 feeder cells/well, in T lymphocyte culture medium supplemented with con A (1 μg/ml). The cells were cultured for 6–7 days at 37°C in 5% CO\(_2\) and viable clones were scored by microscopic inspection. The determination of the clone forming ability and hprt mutant frequency as well as the statistical analysis were performed as described previously (Tates et al., 1994).

**Results**

BNU-induced cytotoxicity in Chinese hamster ovary cell lines AA8 and UV5

The NER-deficient Chinese hamster ovary cell line UV5 and its parental cell line AA8 both lack AGT activity, which makes them suitable to determine unequivocally whether NER acts on BNU-induced lesions. NER-proficient AA8 and NER-deficient UV5 cells were exposed to different doses of BNU and assayed for induced cytotoxicity by measurement of clonal survival (Figure 1). UV5 cells were more sensitive to BNU-induced cytotoxicity than AA8 cells: the D\(_{10}\) value for UV5 (5.2 mM BNU) was 1.4-fold lower than the D\(_{10}\) value for AA8 (7.5 mM BNU) cells. These results suggest that BNU
induces cytotoxic lesions that are a substrate for NER in AGT-deficient cells.

**BNU-induced cytotoxicity in normal and XPA-deficient primary fibroblasts**

Several studies have suggested that NER and AGT cooperate in the removal of cytotoxic DNA damage induced by the ethylating agent N-ethyl-N-nitrosourea (ENU) (Bronstein et al., 1991). To test if this also holds for DNA damage induced by BNU, MEFs from XPA (XPA/−), heterozygote (XPA+/−) and normal (XPA+/+) mice were exposed to BNU (0, 4, 8, 16 and 32 mM) and tested for cell survival using an assay that detects the activity of mitochondrial enzymes of viable cells (Figure 2). XPA MEFs were much more sensitive to BNU-induced cytotoxicity than normal MEFs. The D37 value of XPA MEFs (6 mM BNU) was 2.7-fold lower than the value for normal MEFs (16 mM BNU).

In addition to MEFs, we also tested BNU-induced cytotoxicity in normal and XP-A human fibroblasts, since human and mouse AGT differ in substrate specificity (Liem et al., 1994; Kawate et al., 1995). As already shown for MEFs, XP-A human fibroblasts were also more sensitive to BNU-induced cytotoxicity than normal human fibroblasts (Figure 2), suggesting that the XP-A protein plays an important role in protecting human and mouse cells against the cytotoxic effects of BNU.

**BNU-induced gene mutations in Chinese hamster ovary cell lines AA8 and UV5**

To study the role of NER in protecting AGT-deficient cells against BNU-induced mutagenesis, AA8 and UV5 cells were exposed to different doses of BNU and the induction of mutations was assayed at the hprt locus. hprt mutants were induced with a 1.3-fold higher frequency in NER-deficient UV5 cells than in normal AA8 cells (Table I), suggesting that BNU-induced mutagenic lesions are a substrate for NER.

**BNU-induced mutagenicity in normal and XPA mice**

Several studies have indicated that the target cell population for mutation induction in mouse splenocytes in vivo is proficient for AGT (Skopek et al., 1992; Elder et al., 1998). To test whether NER deficiency has an effect on the frequency of BNU-induced hprt mutants under AGT-proficient conditions, normal (C57BL6) mice and XPA littermates were exposed to single doses of BNU (up to 75 mg/kg) and hprt mutant frequencies were determined in splenocytes, isolated 7 weeks after treatment (Table II). A dose-dependent increase in hprt mutant frequencies was found in splenocytes of normal mice at all exposure levels tested. A similar experiment in 129/ola mice showed a dose-dependent increase in hprt mutant frequency even up to 200 mg/kg BNU (data not shown). A dose-dependent increase in hprt mutant frequency was also found for XPA mice at exposure levels of 25 and 35 mg/kg BNU. These mutant frequencies were 2- to 3-fold higher than those found for normal mice. No difference, however, was found in the hprt mutant frequency in XPA and normal mice when exposed to 75 mg/kg BNU (Table II). At this exposure level, the hprt mutant frequency in XPA mice was markedly lower than that found in mice exposed to 35 mg/kg BNU.

**Discussion**

In this study we investigated the role of mammalian NER in alleviating the cytotoxic and mutagenic effects induced by the direct acting butylating agent BNU in cultured CHO cell lines and in mouse and human fibroblasts as well as in mouse splenocytes.

**BNU-induced cytotoxicity**

The results obtained with AGT-deficient CHO cells revealed a moderate protection by NER against the cytotoxic effects of BNU, since this butylating agent was 1.4-fold more cytotoxic in NER-deficient UV5 cells when compared with the parental.

**Table I. hprt mutant frequencies in CHO AA8 and UV5 cells exposed to BNU**

<table>
<thead>
<tr>
<th>BNU exposure (mM)</th>
<th>AA8</th>
<th>UV5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.23 ± 0.06 x 10^-5 b</td>
<td>1.87 ± 0.23 x 10^-5 c</td>
</tr>
<tr>
<td>2</td>
<td>41 ± 3.6 x 10^-5 c</td>
<td>53 ± 6.6 x 10^-5 c</td>
</tr>
<tr>
<td>4</td>
<td>80 ± 7.9 x 10^-5 c</td>
<td>102 ± 5.6 x 10^-5 c</td>
</tr>
</tbody>
</table>

*Data from three independent experiments.

**Panel A** above shows the cytotoxic effects on normal and XP-A (open circles) fibroblasts were treated with increasing doses of BNU and assayed for cell survival using an assay that detects cell survival. The mean values of three independent experiments with the error bars representing the standard deviation are shown.

**Panel B** above shows the cytotoxic effects in normal and NER-deficient hamster cells exposed to BNU. NER-proficient AA8 (circles) and NER-deficient UV5 (triangles) CHO cells were exposed to increasing doses of BNU and assayed for clonal cell survival. The mean values of four independent observations are shown.
NER-proficient AA8 cells. Hence, in AGT-deficient hamster cells cytotoxic lesions induced by BNU are a substrate for NER and NER does not require AGT to remove these lesions. Recently, it was shown that, in contrast to BNU, the ethylating agent ENU was equally cytotoxic in UV5 cells compared with AA8 cells (Op het Veld et al., 1997). Similar results were found for the methylation agent N-methyl-N-nitrosourea (data not shown). This suggests that cytotoxic butyl lesions are a better substrate for NER compared with methyl or ethyl lesions. It should be mentioned that the UV5 cell line was derived from AA8 cells mutated with ethyl methanesulfonate (Thompson et al., 1980) and therefore UV5 cells may carry mutations in genes other than the ERCC-2 gene. Although the NER defect in UV5 cells is complemented following transfection with the ERCC-2 gene (Weber et al., 1988; Cullinane et al., 1997), it cannot be excluded that the small differences found between AA8 and UV5 cells are caused by a repair defect other than NER.

NER also had an effect on BNU-induced cytotoxicity in mouse and human fibroblasts. BNU was 2.7-fold more toxic in XPA MEFs compared with normal MEFs. It remains to be determined which cytotoxic butyl lesions are substrates for NER and whether AGT is involved in their removal. It has been shown that V79 cells overexpressing the E.coli ogt gene are significantly more resistant to the cytotoxic effects of BNA than normal V79 cells (Harris and Margison, 1993), suggesting that O\(^{6}\)-n-butylguanine is a cytotoxic lesion. It is not very probable, however, that AGT plays an essential role in protecting human fibroblasts against the cytotoxic effects by removing O\(^{6}\)-n-butylguanine. Human AGT has a strong preference for O\(^{6}\)-methylguanine (Liem et al., 1994) and probably requires functional NER to remove O\(^{6}\)-ethylguanine (Bronstein et al., 1991). In addition, O\(^{6}\)-n-butylguanine is efficiently removed in human fibroblasts that lack AGT, whereas the same lesion is very persistent in human cells lacking NER activity (Boyle et al., 1986b). In mouse cells, however, AGT could play a protective role against cytotoxic butyl lesions. Mouse AGT recognizes both O\(^{6}\)-methylguanine and O\(^{2}\)-methylthymine (Kawate et al., 1995) and may exhibit the same substrate specificity as rat AGT, which removes, apart from O\(^{6}\)-methylguanine, O\(^{6}\)-ethylguanine, O\(^{2}\)-propylguanine and even O\(^{6}\)-n-butylguanine (Morimoto et al., 1985).

**BNU-induced mutagenesis**

Alkylation at oxygen atoms of the bases in DNA is generally believed to result in the formation of mutagenic DNA lesions (Swann, 1990). Therefore, since BNU butylates oxygen atoms at guanine and thymine leading to the formation of O\(^{6}\)-n-butylguanine, O\(^{4}\)-n-ethylthymine and O\(^{2}\)-n-butylthymine, it is tempting to propose these lesions as mutagenic DNA damage induced by BNU. A positive correlation between NER activity in cells and protection against BNU-induced mutagenesis would imply that O\(^{6}\)-n-butylguanine, O\(^{2}\)-n-ethylthymine and/or O\(^{2}\)-n-butylthymine are substrates for NER. The present study shows that NER protects AGT-deficient CHO cells to a small extent against BNU-induced mutagenesis, since BNU was 1.3-fold more mutagenic in XPD-deficient UV5 cells compared with NER-proficient AA8 cells. This finding suggests that mutagenic butyl lesions are indeed substrates for NER. O\(^{6}\)-n-butylguanine could be one of these lesions, since: (i) O\(^{6}\)-n-butylguanine induces GC→AT transitions (Chambers, 1991; Baumgart et al., 1993); (ii) O\(^{6}\)-n-butylguanine is efficiently removed in V79 hamster cells via a repair pathway independent of AGT (Boyle et al., 1986a).

A 2- to 3-fold difference in hprt mutant frequency was found between splenocytes of normal and XPA mice exposed in vivo to doses of up to 35 mg/kg BNU. This difference is higher than that found for CHO cells and is probably not due to the fact that the NER deficiency in the two cell types is caused by defects in different genes. The NER deficiency in mouse splenocytes is caused by a lack of the damage recognition protein XPA, whereas NER deficiency in CHO UV5 cells is due to a mutation in XPD. The latter is a helicase and an essential component of the basal transcription factor TFIH (Friedberg et al., 1995). Both mouse XPA embryonic fibroblasts and CHO UV5 cells are almost completely deficient in unscheduled DNA synthesis following exposure to UV light (Zdzienicka et al., 1988; De Vries et al., 1995), indicating that NER activity is abolished in these cells. It should be noted, however, that studies on ENU- or methyl methanesulfonate-induced mutations in mouse splenocytes (Skopek et al., 1992; Elder et al., 1998) suggest that the target cell population for mutation induction is probably proficient for AGT. Although studies using hamster and human cells suggest that O\(^{6}\)-n-butylguanine is mainly removed via an AGT-independent repair pathway (Boyle et al., 1986), it is possible that AGT enhances the removal of this lesion by NER. Thus, analogous to a model for the removal of O\(^{6}\)-ethylguanine in human cells (Bronstein et al., 1991), it could well be that in normal mouse cells AGT and NER cooperate in efficient repair of mutagenic O\(^{6}\)-n-butylguanine, whereas in XPA cells AGT alone is not...
capable of removing O6-butylguanine efficiently. The larger
capability of BER on BNU-induced mutagenesis in AGT-proficient
mouse splenocytes compared with AGT-deficient CHO cells suggests
that BER-mediated removal of mutagenic butyl lesions depends on AGT. Direct evidence for such a mechanism could be obtained by studying the effect of BER on BNU-induced mutagenesis in mice deficient for AGT. Currently, experiments are in progress to determine at the DNA sequence level spectra of BNU-induced hprt gene mutations in normal and BER-deficient mice to analyse the nature of the responsible butyl lesions in mutation induction by BNU.

In contrast to lower exposure levels of BNU, no difference in hprt mutant frequency was found between splenocytes of wild-type and XPA mice treated in vivo with 75 mg/kg BNU. A possible explanation could be that such exposure to BNU proliferation of XPA cells is delayed compared with repair-proficient cells due to the amount of DNA damage. Since rodent lymphocytes are believed to migrate from sites in the body where mutation fixation occurs to the spleen (Jansen et al., 1996), a delay in cell proliferation may affect the period of time between mutation fixation in target cells and repopulation of the spleen. This so-called expression period may be suboptimal in XPA mice exposed to BNU and therefore a lower mutant frequency can be found than one should expect. In normal mice no or less delay in proliferation of target cells will occur due to efficient repair of DNA damage, since an increase in hprt mutant frequency can be found for exposure levels of up to 200 mg/kg BNU.

In conclusion, the present study demonstrates a role of BER in protecting mammalian cells against cytotoxic and mutagenic effects induced by the alkylating agent BNU and suggests that BNU is a suitable model agent to study the mechanisms by which chemicals giving bulky DNA lesions induce genotoxic and carcinogenic effects in mammalian cells.

Acknowledgement
This study was supported by a grant from the Dutch Foundation for Scientific Research (900-501-093).

References
from the DNA of control and repair deficient fibroblasts. Biochem. Biophys.
from the DNA of control and repair deficient fibroblasts. Biochem. Biophys.

References
from the DNA of control and repair deficient fibroblasts. Biochem. Biophys.
from the DNA of control and repair deficient fibroblasts. Biochem. Biophys.

References
from the DNA of control and repair deficient fibroblasts. Biochem. Biophys.
from the DNA of control and repair deficient fibroblasts. Biochem. Biophys.
S.A.M.Bol et al.


Received on October 9, 1998; accepted on January 27, 1999