O6-Methylguanine-DNA Methyltransferase in Human Fetal Tissues: Fetal and Maternal Factors

Steven M. D'Ambrosio, Mervyn J. Samuel, Tapu A. Dutta-Choudhury, and Altaf A. Wani

Departments of Radiology [S. M. D., A. A. W.], Pharmacology [S. M. D., T. A. D-C], and Obstetrics and Gynecology [M. J. S], The Ohio State University Medical Center, Columbus, Ohio 43210-1214

ABSTRACT

O6-Methylguanine methyltransferase (O6-MT) was measured and compared in extracts of 7 human fetal tissues obtained from 21 different fetal specimens as a function of fetal age and race and of maternal smoking and drug usage. Liver exhibited the highest activity followed by kidney, lung, small intestine, large intestine, skin, and brain. Each fetal organ homogenate exhibited a 3- to 5-fold level of interindividual variation of O6-MT. There did not appear to be any significant differences of O6-MT compared in extracts of 7 human fetal tissues obtained from 21 different ABSTRACT

INTRODUCTION

The mutagenic and carcinogenic properties of simple alkylating agents such as environmentally occurring N-nitroso compounds have been demonstrated in a number of systems [reviewed by Lawley (1), Pegg (2), and Singer (3)]. Organ susceptibility to alkylating agents has been correlated with the level and persistence of O-MeGua and the rate of DNA replication (4–9). The O-MeGua adduct in DNA has ambiguous base pairing properties and has been shown to mispair during DNA replication (10–12). O-MT activity has been observed in various human cell types (19) including human lymphocytes (16, 20, 21), skin fibroblasts (17, 22–25), and tumor cells in culture (26, 27) and in extracts obtained from both human adult (28–30) and fetal organs (30, 31). While the levels of O-MT vary widely, as expected, in the adult human tissues (32), Krokan et al. (31) using fetal tissues obtained from common commercial sources. To whom requests for reprints should be addressed, at Division of Radiobiology, Room 103 Wiseman Hall, 400 W. 12th Avenue, Columbus, OH 43210-1214.

The abbreviations used are: O-MeGua, O-methylguanine; O-MT, O-Methylguanine-DNA methyltransferase; MNU, N-methyl-N-nitrosourea. cultured human cells (19, 20) indicate that the O-MT is inducible following chronic exposure to low levels of methylating agents and hepatotoxins. Other studies (37–41) indicate that O-MT is not inducible in human cells. Since the enzyme undergoes suicide inactivation, exposure to a large single dose of alkylating agent may inactivate the repair system until new O-MT can be synthesized. Other factors such as race, age, sex, and exogenous factors such as cigarette smoking, medication, and exposure to occupational and environmental chemicals could also affect the levels of O-MT and account for the large level of individual variation observed within the human population. In order to determine whether a relationship existed between individual O-MT levels and these exogenous and endogenous factors, we quantitated and compared the levels of O-MT in human fetal tissues obtained from 20 nonmedically and 1 medically elected suction curettage. We find: (a) higher O-MT levels for liver and larger interindividual variation than reported previously (31) in human fetal tissue; (b) little if any modulation of O-MT by maternal smoking and fetal age and race; and (c) an increase in O-MT by prenatal exposure to phenoxybenzyl and xenobiotics and drugs accumulated due to poor renal clearance.

MATERIALS AND METHODS

Chemicals. Calf thymus DNA was obtained from Aldrich Chemicals. Dithiothreitol, glycerol, spermidine, and Tris buffer were purchased from Sigma Chemical Co. [3H]MNU (5.4 Ci/mmol) was purchased from New England Nuclear. Calf thymus DNA (10 mg; 4 mg/ml) in N-(2-hydroxyethyl)piperazine-N'-3-propanesulfonic acid buffer (pH 8.0) was treated with 2 mCi of [3H]MNU for 4 h at 37°C. The DNA was dialyzed against 10 mM Tris-HCl (pH 8.0)–100 mM NaCl–1 mM EDTA until no more radioactivity was detected in the dialysate. The methylated DNA was heated at 75°C for 18 h to release 7-methylguanine and 3-methyladenine. This DNA was dialyzed against Tris-EDTA-NaCl to remove the heat released alkylated purines. This DNA had a specific activity of 1.4 × 10⁵ dpm/mg DNA and contained 9 pmol of O-MeGua/mg DNA. All other chemicals were of reagent grade and obtained from common commercial sources.

Human Fetal Tissue. Human fetal brain, liver, lung, kidney, skin, and small and large intestinal tissues were obtained from suction curettings performed for socioeconomic and not medical reasons, except one which was diagnosed as having Down's syndrome. Information concerning the smoking habits, medications, age, and race were obtained from direct interview and patient's chart. Fetal age was determined by the measurement of appendages according to Potter and Craig (42). Immediately following surgery, the tissues were placed into sterile Hanks' balanced salt solution supplemented with 200 μg of streptomycin, 200 units of penicillin, and 500 μg of Fungizone. The tissues were stored at 4°C until processed further usually within 3 h. There was no detectable change in the O-MT activity in tissues stored under these conditions to those not stored in antibiotics. Also, O-MT activity was identical in tissue extracts prepared from freshly isolated tissue or tissue stored at 4°C for 3 h. Tissues collected in this manner maintained their structural and functional integrity as determined by: (a) electron microscopy; and (b) cell culture. Greater than 90% of the cell types isolated from tissues isolated in this manner were viable as determined by trypan blue dye exclusion and growth in continuous
culture (43). The tissues were dissected free of mesenteric tissues, washed with buffer A [50 mM Tris-1 mM EDTA-1 mM dithiothreitol-10% (v/v) glycerol, pH 7.8], and stored in 2 volumes of this buffer at –70°C.

Preparation of 9000 \times g Supernatants. Tissues were thawed on ice. Measurement of a wet weight provided the basis for tissue concentration (25%, w/v) during the homogenization procedures to assure uniformity in further subfractionation of the tissue. Tissues were minced to small pieces with a pair of surgical scissors in buffer A. The brain, large intestinal, liver, lung, kidney, and small intestinal tissues were homogenized with 6 parts of an electrically driven tight fitting pestle of a Potter-Elvehjem Teflon homogenizer in the above buffer chilled on ice as described previously (30). Few, if any, unbroken cells or nuclei were detected by phase contrast microscopy. The crude homogenates were centrifuged twice at 800 \times g for 20 min at 4°C. The S9 fraction was then prepared by centrifugation at 9000 \times g for 20 min at 4°C. The supernatant was clarified by a second centrifugation at 9000 \times g. The underlying s.c. tissue of the skin was scraped free with a surgical scalpel blade prior to measurement of wet weight. The skin was then minced to approximately 1-mm³ pieces with a pair of scissors. The tissue suspension was initially disrupted by six 15-s bursts of a Tektron homogenizer with 30 s of cooling on ice between each burst. This crude homogenate was further disrupted by a Teflon homogenizer and subfractionated to prepare 9000 \times g supernatant.

The tissue 9000 \times g supernatants were divided into 500-μl aliquots and stored in the buffer A at –70°C after quick freezing in liquid nitrogen. The aliquots were subsequently used for protein and O'-MT determinations. Our data and those reported by others (28) indicate that the O'-MT being quantitated is stable under these conditions of storage.

Quantitation of O'-MT. Transfer of the radiolabeled methyl group from the O6-position of guanine in DNA to the transferase protein was quantitated by measuring the radioactivity solubilized by subsequent treatment with proteinase K (16). The method was validated using the high performance liquid chromatography quantitation of O'-methylguanine following the hydrolysis of alkylated DNA after incubation with the tissue homogenates. Identical values for O'-MT were observed using either the indirect or direct method of determination (30). The protein concentration of each tissue S9 homogenate was determined by the method of Bradford (44). Increasing protein concentrations (0 to 600 μg) of each sample were incubated with 15 μg of the heat treated radiolabeled alkylated DNA substrate in a total assay volume of 0.5 ml of buffer A containing 50 μM spermidine. The samples were incubated with agitation at 37°C for 45 min. To one set of samples were added 100 μg of proteinase K and 0.5 μg of sodium dodecyl sulfate and the incubation at 37°C was continued for 60 min. At the end of this incubation, the DNA was precipitated from all samples by the addition of 100 μg of carrier calf thymus DNA, NaCl (0.15 M final concentration), and 2 volumes of 1% trichloroacetic acid in 80% ethanol and stored at –20°C for 30 min (29, 31). The samples were then centrifuged at 5000 \times g for 20 min and a 1.0-ml sample of the supernatant was counted for radioactivity using Beckman’s Ready-Solve. Nonspecific degradation of substrate was found never to exceed 5%. The specific activity of O'-MT in pmol/mg protein was calculated by [dpm (protease treated) – dpm (non-protease treated)] divided by the specific activity of the [3H]MNU. The determinations from several protein concentrations in the linear range were normalized to 1 mg of protein and the averages expressed.

RESULTS

Maternal and Fetal Factors. The concentration of O'-MT was measured in the S9 fractions of homogenates from human fetal liver, kidney, lung, small intestine, large intestine, skin, and brain tissues. All experiments were performed double blind. O'-MT activities for each of the tissues was first measured and then correlated to maternal-fetal information. Information concerning the age and race of the fetus and the medication and smoking history of the mother during pregnancy is given in Table 1. The gestation period of the fetuses was between 16 and 22 weeks with a mean age of 18.2 ± 1.7 weeks. A majority (15 of 21) of the fetuses were Caucasian, 4 were Negroes, and 2 were of an unknown background. Of the 21 mothers, 4 were found to be cigarette smokers, 14 were nonsmokers, and information was not available on 3 mothers. Within the smokers’ group, 1 individual smoked 6 packs of cigarettes per week, while the other 3 smoked only 1 to 2 packs per week. Individual J was taking 100 mg of phenobarbital per day. This individual also smoked 1–2 packs of cigarettes per week. Individual G was on kidney dialysis, but the information on the drugs being used was not available to us. Individual Q was using antihistamines for allergies but the precise identity of the drugs was also not available. Fetus U was diagnosed as having Down’s syndrome, while all others were apparently normal.

O'-MT Activity as a Function of Tissue. The amount of O'-MT in each tissue is shown as a comparative distribution in Fig. 1. Inspection of the distribution of O'-MT activity for each tissue indicated a consistent pattern of variation between individuals. The majority of individuals appeared to cluster around one mean with some individuals exhibiting significantly, i.e., 2 SD above the mean, higher values. In most cases single individuals had all their tissue O'-MT values segregated into either of these groups. This variation noted between individuals could not be due to experimental variation. Variation within the assay for triplicate samples was approximately 5%. Some tissue samples were also tested at different storage times at –70°C during the course of this study and were found to vary only 7%.

Interpretation of the results in Fig. 1 are subject to limitations imposed by a statistically small number of individuals. Because a normal population distribution could not be assumed, comparison between tissues was assigned on the basis of nonparametric two way analysis of variance. Friedman’s test was used to analyze the significance of the difference in values between tissues. O'-MT values of individuals G and J were excluded from this group because of their relative divergence from the other tissue values and their known history of medication. Based on this test (P < 0.001) liver had the highest rank order for O'-MT followed by kidney, lung, small intestine, large intestine, skin, and brain. The mean values of O'-MT together
O'-MT IN HUMAN FETAL TISSUES

Table 3 Alkyltransferase activity in different human groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver</th>
<th>Kidney</th>
<th>Lung</th>
<th>Small intestine</th>
<th>Large intestine</th>
<th>Skin</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>0.338</td>
<td>0.197</td>
<td>0.219</td>
<td>0.175</td>
<td>0.095</td>
<td>0.061</td>
<td>0.108</td>
</tr>
<tr>
<td>H</td>
<td>0.468</td>
<td>0.242</td>
<td>0.211</td>
<td>0.206</td>
<td>0.076</td>
<td>0.082</td>
<td>0.042</td>
</tr>
<tr>
<td>I</td>
<td>0.233</td>
<td>0.109</td>
<td>0.102</td>
<td>NA</td>
<td>0.115</td>
<td>0.113</td>
<td>0.047</td>
</tr>
<tr>
<td>K</td>
<td>0.663</td>
<td>NA</td>
<td>NA</td>
<td>0.677</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>L</td>
<td>NA</td>
<td>0.121</td>
<td>NA</td>
<td>0.060</td>
<td>0.046</td>
<td>0.105</td>
<td>0.052</td>
</tr>
<tr>
<td>M</td>
<td>0.182</td>
<td>0.160</td>
<td>0.067</td>
<td>NA</td>
<td>0.163</td>
<td>0.188</td>
<td>0.035</td>
</tr>
<tr>
<td>O</td>
<td>0.120</td>
<td>0.026</td>
<td>0.034</td>
<td>NA</td>
<td>0.022</td>
<td>0.025</td>
<td>NA</td>
</tr>
<tr>
<td>P</td>
<td>0.119</td>
<td>0.061</td>
<td>0.057</td>
<td>0.111</td>
<td>0.364</td>
<td>0.042</td>
<td>0.037</td>
</tr>
<tr>
<td>R</td>
<td>0.366</td>
<td>0.145</td>
<td>0.158</td>
<td>0.288</td>
<td>0.201</td>
<td>0.022</td>
<td>0.062</td>
</tr>
<tr>
<td>S</td>
<td>0.291</td>
<td>0.165</td>
<td>0.229</td>
<td>0.269</td>
<td>0.190</td>
<td>0.102</td>
<td>NA</td>
</tr>
<tr>
<td>T</td>
<td>0.483</td>
<td>NA</td>
<td>NA</td>
<td>0.217*</td>
<td>0.217*</td>
<td>NA</td>
<td>0.073</td>
</tr>
<tr>
<td>U</td>
<td>0.140</td>
<td>NA</td>
<td>NA</td>
<td>0.051</td>
<td>0.266</td>
<td>0.083</td>
<td>0.072</td>
</tr>
</tbody>
</table>

1. No smokers, no medication

2. Smokers
   A     | 0.278 | 0.303  | 0.128| NA              | NA              | 0.123| NA    |
   C     | 0.377 | 0.392  | 0.277| 0.217           | 0.101           | 0.115| 0.061 |
   N     | 0.178 | 0.052  | 0.063| 0.084           | 0.061           | 0.032| 0.025 |

3. Medications
   G     | 0.687 | 0.520  | 0.437| NA              | NA              | 0.393| NA    |
   J     | 1.540 | 0.800  | 0.725| 0.860           | 0.750           | 0.565| 0.101 |
   Q     | 0.406 | 0.253  | 0.087| 0.227*          | 0.227*          | NA   | 0.065 |

4. Unknown
   D     | NA    | NA     | NA   | NA              | NA              | 0.145| NA    |
   E     | 0.616 | 0.272  | 0.196| 0.130           | 0.082           | 0.103| 0.103 |
   F     | 0.776 | 0.405  | 0.266| 0.116           | 0.098           | 0.138| 0.120 |

* Groups are described according to the maternal information listed in Table 1.

Fig. 1. Distribution of O'-MT in human fetal liver, kidney, lung, small and large intestine, skin, and brain obtained from a population consisting of 21 individuals. The specific activity in the S9 tissue homogenates is expressed as pmol O'-MT/mg protein. The designated letter codes are described in Table 1.

Table 2 Mean values of O'-methylguanine DNA methyltransferase in tissue S9

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Sample set</th>
<th>n</th>
<th>(Mean ± SE)</th>
<th>Rank sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>ABCFHIKMNOPQRSTUVWXYZ</td>
<td>17</td>
<td>0.405 ± 0.052</td>
<td>54.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>ABCFHIKMNQPSRSTU</td>
<td>15</td>
<td>0.221 ± 0.035</td>
<td>40</td>
</tr>
<tr>
<td>Lung</td>
<td>ABCFHIKNPSQRRST</td>
<td>14</td>
<td>0.181 ± 0.022</td>
<td>38</td>
</tr>
<tr>
<td>Small intestine</td>
<td>BCFHIKNPQQRST</td>
<td>13</td>
<td>0.166 ± 0.019</td>
<td>35</td>
</tr>
<tr>
<td>Large intestine</td>
<td>BFCHIKMNOPQRSTUVWXYZ</td>
<td>16</td>
<td>0.135 ± 0.025</td>
<td>24</td>
</tr>
<tr>
<td>Skin</td>
<td>ABCFHIKNOPSUR</td>
<td>14</td>
<td>0.074 ± 0.011</td>
<td>18.5</td>
</tr>
<tr>
<td>Brain</td>
<td>ABCDEFGHIKNMOPQRSTUVWXYZ</td>
<td>17</td>
<td>0.069 ± 0.009</td>
<td>14.5</td>
</tr>
</tbody>
</table>

with the statistical parameters are given in Table 2.

Age and Race. There was very little variation in the gestation age of the fetuses (Table 1). Therefore, no conclusive relationship between the fetal age and O'-MT activity could be made. Four of the fetuses were Negro leading to consideration of race as a factor with respect to the larger number of Caucasians. Analysis of data points chosen on the basis of race by Friedman's analysis indicated that no significant difference was discernible. The means for the Negro group were: liver, 0.331 ± 0.050 (SE), n = 3; kidney, 0.297 ± 0.098, n = 3; lung, 0.208 ± 0.075, n = 3; small intestine, 0.206 ± 0.019, n = 2; large intestine, 0.098, n = 2; skin, 0.088, n = 2; brain, 0.109 ± 0.036, n = 4.

Smoking. Table 3 categorizes the O6-MT determinations of individuals into four groups: nonsmoker, nonmedicated; smoker; medicated; and unknown. Four individuals smoked regularly during pregnancy. The effects of this on fetal O'-MT levels were compared on an individual basis to both the overall means given in Table 2 and the means for the known non-smoker, nonmedicated group (not given). Individual J was not considered as part of this group since the mother was on drug therapy and the fetal tissue values were significantly different from other individuals who smoked. For the 2 mothers, A and C, who smoked 1 to 2 packs/week, the O6-MT values were within the nonsmoking distribution (Fig. 1). For individual N, who smoked 6 packs/week during pregnancy, the fetal values for O6-MT also were within the same range of O'-MT.

Medication. Three individuals (G, J, Q) were known to be receiving drug therapy. The O6-MT values of these individuals were compared to the other groups (Table 3). Individual G was undergoing kidney dialysis for chronic renal failure during the time of her pregnancy. The identity of drugs received or the level of retained toxins by this individual was not available to us. When compared to other individuals in the nonmedicated, nonsmoker group or the overall tissue means given in Table 2, significantly elevated values were found in kidney (0.520 pmol/mg protein), lung (0.437 pmol/mg protein), and skin (0.393 pmol/mg protein). Liver was the other tissue measured for this individual but was not outside the bounds of normal variation within the data set. Individual J was receiving phenobarbital (100 mg/day). The O6-MT values of all tissues quantitated except brain were found to be significantly (P < 0.005) elevated over individuals receiving no medication. O6-MT in liver (1.540 pmol/mg protein), kidney (0.800 pmol/mg protein), lung (0.725 pmol/mg protein), small intestine (0.860 pmol/mg protein), large intestine (0.750 pmol/mg protein), and skin (0.565 pmol/mg protein) were all separated from the overall mean values of the tissues in Table 2 by greater than 4 SD. Levels in brain tissue for individual J were similar to values obtained from the other groups in Table 3. Individual Q was receiving antihistamines for severe allergies during pregnancy. The O'-MT values of this individual were similar for all tissues quantitated to values in the nonmedicated group and also to the overall mean values.

53
O6-MT IN HUMAN FETAL TISSUES

Down's Syndrome and Unknown Individuals. One individual, U, was diagnosed as having Down's syndrome. The values of O6-MT for each of the tissues measured were within the range of observed variation within the data set and therefore could not be considered significantly different. Patient history was not available for individuals D, E, and F. The O6-MT values for these individuals (Table 3) when compared to the other groups could not be considered significantly different since tissue values were within about 1 SD of the overall tissue means (Table 2).

DISCUSSION

In this study we quantitated and compared the levels of O6-MT in 7 different human fetal tissues obtained from 21 different fetal specimens as a function of fetal age and race and maternal smoking and drug use. We find that liver exhibits the highest level of O6-MT followed by kidney, lung, small intestine, large intestine, skin, and brain. The difference between the levels of O6-MT in the liver and brain was 10-fold. Each of the tissues exhibited a 3- to 5-fold interindividual variation in the level of O6-MT. Fetal race and age and maternal smoking did not appear to modulate the activity of the O6-MT. The fetal specimens obtained from one individual on phenobarbital exhibited a significantly ($P < 0.005$) 4-fold higher increase in O6-MT activity, suggesting that phenobarbital might induce O6-MT in humans in vivo. The tissues obtained from one mother with renal failure and undergoing kidney dialysis were also increased 2- to 3-fold.

The O6-MT values for human fetal liver obtained from elective abortions and using the direct assay are similar to that observed using high performance liquid chromatography analysis (30) and are 3-fold higher than reported previously (31) using spontaneously aborted tissue and the direct assay. Our human fetal liver O6-MT values are similar to those reported previously in human adult liver by us (30) and by Yarosh et al. (45) and one-half that reported by two other groups (15, 28, 29). The values obtained for fetal large intestine are somewhat lower, while fetal kidney, lung, and brain are close to those reported (31). Our values for fetal brain and large and small intestine are similar to those reported in the corresponding adult tissues (29, 46). These differences noted in the fetal O6-MT activities probably reflect the source of tissue, i.e., spontaneous versus elective abortions.

Individual DNA repair activities in adult human tissue extracts vary severalfold (32). This wide variation could represent either genetic diversity of the population or the inducibility of the repair enzymes in individuals exposed to environmental agents. However, a previous study on human fetal O6-MT indicated very little individual variation suggesting a genetic homogeneity with respect to O6-MT. Our study utilizing a much larger number of individuals (21 versus 5 fetuses) and tissue obtained from elective rather than spontaneous abortions indicated a rather wide interindividual variation in the O6-MT levels similar to that observed in the adult population. In order to gain further insights into factors which might modulate this repair enzyme in vivo, we examined the relationship between fetal race, age, and Down's syndrome and maternal smoking, drug use, and health on the level of O6-MT in the human fetal organs. Within the limited sample size studied, there appeared to be little, if any, correlation with the individual fetal levels of O6-MT and fetal race, fetal age, maternal smoking, or Down's syndrome. These data would support the hypothesis that the wide individual variation observed with O6-MT in the fetal and adult human population is due primarily to the genetic diversity of a highly homogeneous population.

O6-MT is induced in the rat liver following treatment with alkylating agents like dimethyl- and diethylnitrosamine and 1,2-dimethylhydrazine but not by the direct acting alkylating agents, streptozotocin and MNU (37, 47). Rat liver O6-MT is also induced by the hepatotoxins, thioacetamide, carbon tetrachloride, phenobarbital, aflatoxin, and acetylaminofluorene (37, 47, 50). A few studies (19, 20, 39, 40) with human cells in culture provide somewhat contradictory data on whether O6-MT is inducible. This induction may be related to a protective process by cells to counteract the accumulation of the potentially hazardous miscoding lesion O-alkylguanine (2, 30, 48). However, the apparently normal levels of O6-MT in the fetal tissues obtained from the mothers who smoked cigarettes may suggest a lack of induction by nitrosamines present in $\mu$g quantities in tobacco products and smoke (49). In two individuals, the levels of O6-MT were significantly ($P < 0.005$) higher than the mean of remaining population. The most striking difference in these two individuals was that the mother was either exposed to daily doses of phenobarbital for treatment of epilepsy or undergoing kidney dialysis for treatment of renal failure. A study (50) in rats pretreated for 8 weeks with phenobarbital indicated higher levels of O6-MeGua repair in the livers of treated compared to nontreated rats. This induction was not related to hepatocellular proliferation. It is interesting to note that O6-MT was increased in human fetal tissues other than liver, indicating a general organ response. Thus it is possible that phenobarbital, which does not damage DNA, acts by an as yet unknown process in vivo to enhance O6-MT. Renal failure greatly interferes with the clearance and elimination of drugs and other xenobiotics. As a result, the xenobiotics or their metabolites can accumulate and be retained for long periods of time. Furthermore, patients undergoing dialysis routinely receive drugs for renal associated complications. The observed enhancement of O6-MT in many of the fetal tissues tested could be the result of the retention of toxic xenobiotics or drugs. Such chronic exposure could act directly to increase the synthesis of O6-MT or indirectly through increased cellular replication. However, further studies are required to determine whether these increased levels of O6-MT were unique to these two individuals or result of environmental induction. In summary, it is clear from our and other (30, 31) studies that the human fetus is capable of repairing the O6-MeGua adduct in DNA and synthesizing O6-MT.

ACKNOWLEDGMENTS

We wish to thank Ruth Gibson-D'Ambrosio for the procurement of the tissue.

REFERENCES


20. Waldstein, E. A., Cao, E. H., and Setlow, R. B. Adaptive resynthesis of O6-

deoxyribonucleic acid in vivo in various organs of C57BL mice by the
carcinogens N-methyl-N-nitrosourea, N-ethyl-N-nitrosourea and ethyl meth-
anesulfonate in relation to induction of thymic lymphomas. Biochem. J.,


dose-response relationships for mammalian tumor induction by a single admin-

24. Toorchen, D., and Topal, M. D. Mechanisms of chemical mutagenesis and
carcinogenesis: effects on DNA replication of methylation at the O'-guanine
residues in DNA by mammalian liver extracts. Carcinogenesis (Lond.), 4:
1599-1584, 1983.

25. Proctor, R. B. Variations in DNA repair among humans. In: C. C. Harris and
H. N. Aurbach (eds.). Human Carcinogenesis, pp. 231-254. New York:

residues in DNA by mammalian liver extracts. Carcinogenesis (Lond.), 6:

27. Day, R. S. Ill, Yarosh, D. B., and Ziolkowski, C. H. J. Relationship of


29. Wani, A. A., Wani, G., and D'Ambrosio, S. M. Repair of DNA O6-alkylation

30. Krokan, H., Haugen, A., Myrnes, B., and Guddal, P. H. Repair of premuta-
genic DNA lesions in human fetal tissues: evidence for low levels of O6-
methylguanine-DNA methyltransferase and uracil-DNA glycosylase activity
in some tissues. Carcinogenesis (Lond.), 4: 1559-1564, 1983.

31. Setlow, R. B. Variations in DNA repair among humans. In: C. C. Harris and
H. N. Aurbach (eds.). Human Carcinogenesis, pp. 231-254. New York:

residues in DNA by mammalian liver extracts. Carcinogenesis (Lond.), 6:

33. Hall, J. H., Bresil, H., and Montesano, R. O6-Alkylguanine DNA alkyltrans-
ferase activity in monkey, human and rat liver. Carcinogenesis (Lond.), 6:

34. Pegg, A. E., West, L. F., Foote, R. S., Mitra, S., and Perry, W. Purification
and properties of O6-methylguanine-DNA transmethylase from rat liver. J.

35. Fedoroff, V., Margison, G. P., and O'Connor, P. J. Mechanisms of carcino-
genesis induced by alkylating agents. Biochim. Biophys. Acta, 823: 111-145,
1985.

36. Pegg, A. E., and Perry, W. Stimulation of repair of methyl groups from
O6-methylguanine DNA to protein in rat liver extracts in response to

37. Foote, R. S., Mitra, S., and Pal, B. C. Demethylation of O6-methylguanine
in a synthetic DNA polymer by an inducible activity in Escherichia coli.
1980.

38. Karran, P., Arlett, C. F., and Broughton, B. C. An adaptive response to the
cytotoxic effects of N-methyl-N-nitrosourea is apparently absent in normal

39. Krokan, H., Lechner, J., Krokan, R. H., and Harris, C. C. Normal human
bronchial epithelial cells do not show an adaptive response after treatment
with N-methyl-N-nitro-N-nitrosoguanidine. Mutat. Res., 146: 205-209,
1985.


41. Gibson-D'Ambrosio, R. E., Leong, Y., and D'Ambrosio, S. M. DNA repair
following ultraviolet and N-ethyl-N-nitrosourea treatment of cells cultured
from human fetal brain, intestine, kidney, liver, and skin. Cancer Res., 43:
5846-5850, 1983.

42. Bradford, M. A rapid and sensitive method for the quantitation of microgram

43. Yarosh, D. B., Forance, A. J., and Day, R. S., III. Human O6-alkylguanine-
DNA alkyltransferase fails to repair O6-methylthymidine and methyl phos-
phoribosesters in DNA as efficiently as does the alkyltransferase from Esche-

44. Wiestler, O., Kleihues, P., and Pegg, A. E. O6-Alkylguanine-DNA alkyltrans-
ferase activity in human brain and brain tumors. Carcinogenesis (Lond.), 5:

45. Pegg, A. E. Repair of O6-methylguanine in DNA by mammalian tissues. In:
H. Greim, R. Jung, M. Kramer, H. Marquardt, and F. Oesch (eds.). Bio-
chemical Basis of Chemical Carcinogenesis, pp. 265-274. New York: Raven

Repair of O6-methylguanine in human fetal and skin cells in culture.
Carcinogenesis (Lond.), 5: 121-124, 1984.

47. Hoffmann, D., Adkins, J. D., Bruneman, K. D., and Hecht, S. S. Assessment
of tobacco-specific N-nitrosamines in tobacco products. Cancer Res., 39:

48. Den Engelse, L., Kleihues, P., and Pegg, A. E. O6-Alkylguanine-DNA alkyltrans-
ferase activity in human brain and brain tumors. Carcinogenesis (Lond.), 5:

49. Pegg, A. E. Repair of O6-methylguanine in DNA by mammalian tissues. In:
H. Greim, R. Jung, M. Kramer, H. Marquardt, and F. Oesch (eds.). Bio-
chemical Basis of Chemical Carcinogenesis, pp. 265-274. New York: Raven

Repair of O6-methylguanine in human fetal brain and skin cells in culture.
Carcinogenesis (Lond.), 5: 121-124, 1984.

51. Hoffmann, D., Adkins, J. D., Bruneman, K. D., and Hecht, S. S. Assessment
of tobacco-specific N-nitrosamines in tobacco products. Cancer Res., 39:

repair of O6-methylguanine in livers of rats pretreated with phenobarbital
$O^6$-Methylguanine-DNA Methyltransferase in Human Fetal Tissues: Fetal and Maternal Factors


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/47/1/51

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.