Tissue-specific expression of DNA repair gene, N-methylpurine-DNA glycosylase (MPG) in Balb/c mice without external damage

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The N-methylpurine-DNA glycosylase (MPG), a ubiquitous DNA repair enzyme, removes N-methylpurine and other damaged purines induced in DNA. Tissue-specific mRNA levels of the N-methylpurine-DNA glycosylase (MPG) were investigated in Balb/c mice of four different growing stages; newborn, 1, 4 and 8-weeks postpartum. MPG expressions in the newborn and the 8-week-old mice were the highest in thymus and testis, respectively. The tested tissues of the newborn mice had consistently higher MPG mRNA level than 8-week-old adults except in testis and thymus. The MPG mRNA level in testis was the lowest in the newborn mice, but it attained the highest in the 8-week-old mice. The levels of MPG mRNA among the different tissues in the newborn and the 8-week-old mice were more than 9.0 and 19.0-fold respectively. These results suggest that the of MPG expression was dependent on the growing stage and had tissue-specificity.

Keywords: DNA repair, gene expression, mouse, MPG, tissue-specificity

INTRODUCTION

DNA repair is a universal phenomenon that maintains the sequence and structural integrity of genome which are continuously damaged either by spontaneous reactions or by exposure to external physical and chemical agents. The alkyl adducts in DNA may lead to toxic and mutagenic responses (Washington *et al.*, 1989). N-Alkylpurines in DNA may themselves be toxic to the cell (Lindahl *et al.*, 1988; Mitra *et al.*, 1992). The evolution of repair system for alkyl adducts may be related to the fact that even in the absence of exposure to external alkylating agents, spontaneous methylation of various nucleophilic sites in DNA may occur *in vivo* by nonenzymatic reaction with *S*-adenosylmethionine (Barrow and Magee, 1982). Endogenous damage to DNA may be a major contributor to both ageing and cancer (Park and Ames, 1988). N-Alkylpurines in DNA may also contribute to other biological hazards, especially the induction of chromosomal aberrations. There is a correlation between N-alkylation level and clastogenic efficiency of various alkylating agents. Also, the significance of previous studies about the change in the level of alkylation repair in ageing is not clear (Gensler and Bernstein, 1981; Kovacs *et al.*, 1984; Bond and Singh, 1987; Licastro and Walford, 1986; Mullaat *et al.*, 1990; Wei *et al.*, 1993).

N-Methylpurine DNA glycosylase (MPG), a ubiquitous DNA repair enzyme, removes N-methylpurine and other damaged purines induced in DNA. Their glycolytic removal leaves apurinic sites in DNA, which, when not further repaired, could lead to cell death, point mutations, chromosome aberrations, ageing and carcinogenesis (Generoso, 1982; Loeb and Preston, 1986; Samson *et al.*, 1991). It is, therefore, important to investigate the possible tissue-specificity of repair of the alkyl adducts in DNA in detail. *Escherichia coli* has two MPGs: namely, the AlkA and Tag proteins, the products of *alk*A and *tag* genes (Gallangher and Brent, 1984; Chakravarti *et al.*, 1991). The constitutively expressed Tag protein is specific for 3-alkyladenine (Bjelland and Seeberg, 1987). In contrast, the AlkA protein, a component of the *ada* regulon and inducible by alkylating agents, has a broad substrate specificity and removes not only 3-alkyl-adenine, but 7-alkylguanine and 3-alkylguanine as well, and also O^2-methylthymine and O^2-methylcytosine (Lindahl, 1982; McCarthy *et al.*, 1984). However, the mammalians appear to have only one MPG, which is more akin to the AlkA protein in that it removes all three N-methylpurines in

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DNA (Craddock and Henderson, 1982; Gallangher and Brent, 1984; Helland *et al.*, 1987; Chakravarti *et al.*, 1991).

Since the mouse model is being extensively used for mutagenesis of xenobiotic compounds including alkylating agents, it is also important to consider the tissue-specific variation of different growth stages in the mRNA level of DNA repair genes. Regulation of enzyme activity may occur at various levels. According to Washington *et al.* (1988, 1989), the activity of the mouse MPG is regulated in a organ-specific and age-dependent fashion. The repair activities for N-alkylpurine and O^6-methylguanine vary widely in different tissues and the lack of repair of O^6-alkylguanine has been correlated with tumor induction in rats (Pegg, 1984). DNA repair has been reported to decline with age in isolated rodent cells (Park and Ames, 1988; Washington *et al.*, 1989). Chakravarti *et al.* (1991) observed that cells of mouse origin have an intermediate level of MPG message compared to human and rat cells.

Even fewer data are available for the mammalian MPG, thus, the molecular basis of regulation of MPG has not been extensively studied (Engelward *et al.*, 1993; Kim *et al.*, 1997; Roy *et al.*, 1997). Therefore, we have measured the levels of MPG mRNA in various organs at 4 different growing stages without giving external damage.

MATERIALS AND METHODS

Preparation of mouse tissues

Mice were sacrificed periodically at newborn (within 24h after birth) and, 1, 4 and 8 weeks postpartum. The various organs (brain, heart, kidney, liver, lung, muscle, spleen, stomach, testis and thymus) of Balb/c mice were rapidly resected and dipped into liquid nitrogen and stored at -70°C until use for RNA preparation.

Preparation of total cellular RNA and Northern blot hybridization

Total cellular RNA was isolated by the acid phenol method of Chomzynski and Sacchi (1987). The samples were prepared by homogenization from organs of 2-3 animals. 20 μg RNA in each lane of 1.2% agarose gel containing 2.2M formaldehyde was electrophoresed in 1× MOPs buffer and transferred onto nylon membranes by capillary movement in 10x standard saline citrate solution. Filters were hybridized to ^{32}P-labelled DNA probes at 42°C for 14-16 h and washed twice with 2× SSC at room temperature for 5 min, 3 times with 2× SSC/0.05% SDS at 55°C for 15 min, and twice with 0.1× SSC at room temperature for 5 min. The level of MPG expressions was calculated after the scanning of autoradiographic film using laser densitometer.

DNA probe preparation

The mouse MPG cDNA fragment (1.1kb) was prepared by *Eco*RI digestion of the pGI 1945 clone (Tatsuka *et al.*, 1995) and the CHOb insert (0.74kb) was obtained by the digestion of the cDNA clone with *Xho*I-*Eco*RI which were derived from the rat cDNA library (Lim *et al.*, 1994). The fragments were radiolabeled by the method of random priming using α-[^{32}P]dCTP and used as a probe for the Northern blot hybridizations.

RESULTS

Tissue-specific level of MPG mRNA

We surveyed the MPG mRNA expression in 10 organs (brain, heart, kidney, liver, lung, muscle, spleen, stomach, testis and thymus) in Balb/c mice at four different growing

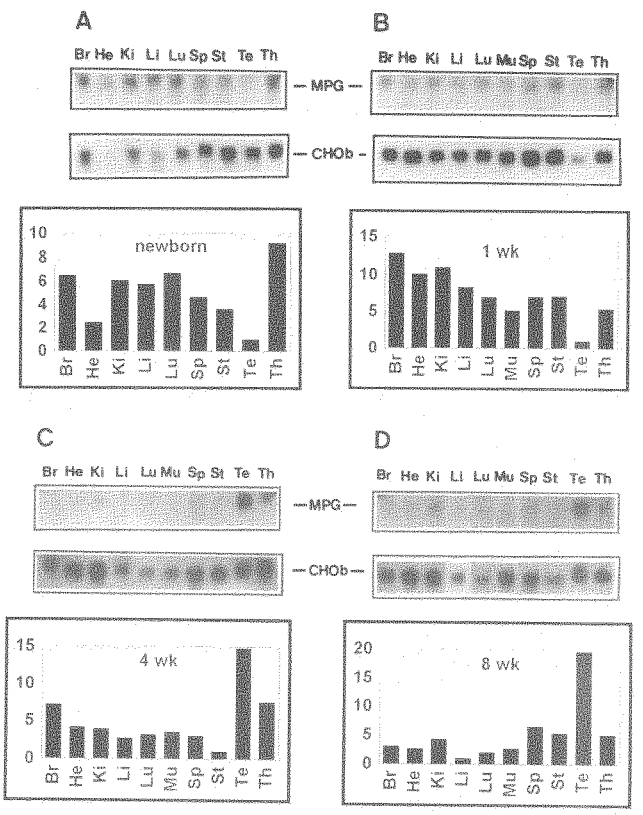

Fig. 1. Expression of MPG gene in newborn, 1 week, 4 weeks and 8 weeks old Balb/c mice. Each blot was hybridized with labeled MPG cDNA (1.1kb) and the relative amount of mRNA was normalized with the level of CHOb (1.3kb) mRNA. Relative mRNA level was calculated by laser dens

stages, such as, newborn, 1, 4, 8-week-old, except the muscles of newborn mice (Fig. 1).

Newborn mice were sacrificed within 24h after birth and expressions of MPG mRNA at the various organs were determined and normalized with that of CHOb. CHOb mRNA was also measured at the same time for a quantitative load of RNA. MPG message of the newborn mice was decreasing in the order of thymus, lung, brain, kidney, liver, spleen, stomach, heart and testis. MPG mRNA in thymus was the highest level in the newborn mice. The message was also detected in the lung, brain, kidney, liver, spleen, stomach and heart. Testis expressed CHOb at very high level in the newborn mice, but the level of MPG mRNA was very low in the organ (Fig. 1A).

MPG expression in the 8-week-old (young adult) mice was decreasing in the order of testis, spleen, stomach, thymus, kidney, brain, muscle, heart, lung and liver. The highest level of MPG mRNA was found in testis of young adults. Also, the expression of MPG in the spleen, stomach, thymus, kidney and brain was detectable in the 8-week-old mice (Fig. 1D). However, in the other organs, namely, skeletal muscle, heart, lung and liver, it showed a very low level of expression in the 8-week-old mice. The MPG mRNA expression was more than 9.0-fold higher in thymus than testis of newborn mice, but the higher level of MPG expression drastically switched in testis from thymus in the 8-week-old mice (Fig. 1A, D). Tissue-specific MPG mRNA levels in the 1-week-old and the 4-week-old mice were similar to newborn and 8-week-old mice (Fig. 1). Interestingly, the brain has the highest level of MPG among the organs in 1-week-old mice (Fig. 1B).

DISCUSSION

In order to understand the tissue-specific regulation of MPG gene at the transcriptional level, we have analyzed changes of MPG expression in mice from newborn through 8-week-old. There were significant tissue-specific differences in the mRNA level of MPG. There were also significant tissue-specific differences in the mRNA expression of MPG. Among the tested organs, the highest MPG expression was found in thymus and testis of newborn and 8-week-old adult mice (Washington *et al.*, 1988, 1989). The level of MPG mRNA among the different tissues in the newborn and the 8-week-old mice was more than 9.0 (e.g., between thymus and testis in newborn mice) and 19.0-fold respectively. The differences between the newborn and the 8-week-old mice in the mRNA level among the different organs were about 2-fold. The eight-week-old adult mice had higher differences among the tissues tested than the newborn mice. The large variation in the MPG mRNA level among different tissues from the same strain underscores the uncertainty in relating the role of DNA repair to mutation induction and human genetic risk, particularly, when the experiments involve different cell types (Washington *et al.*, 1989). However, the significance of tissue-specific variation in the MPG level is not clear. One possible explanation is that the liver DNA may be the target for the highest level of adduct formation, because the active species for alkylation are often produced by P450-mediated metabolic activation of alkylating mutagens and carcinogens (Nebert and Gonzalez, 1987; Washington *et al.*, 1989; Mitra and Kaina, 1993; Bellec *et al.*, 1996). On the basis of this hypothesis, we also expected that the highest level of other alkylation repair proteins would be present in liver. It was, therefore, extremely surprising to observe that in mouse, the MPG mRNA level was highest in the testis of 8-week-old mice, higher than that of the liver, and that the brain had also a high level of expression.

Engelward *et al.* (1993) reported that the highest and lowest MPG mRNA levels were seen in the testes and heart respectively, with a 21-fold difference. Kidney, liver and spleen showed intermediate levels of MPG mRNA and the brain, lung and skeletal muscle levels were almost as low as that seen in the heart. The results were similar to our results except in the liver tissue. One other DNA repair gene, XPBC/ERCC-3, also showed relatively high levels of mRNA in the testes, suggesting that there may be a generally increased level of DNA repair in these germ line cells (Weeda *et al.*, 1991; Engelward *et al.*, 1993).

However, these patterns were different from the other results of MPG activity (S. Mitra, personal communication). The MPG activity was studied in six organs (brain, liver, lung, spleen, stomach, ovary) from 6- to 10-week-old Blab/c mice. The tissues can be arranged in order of decreasing activity of glycolytic removal as stomach > liver > spleen > lung > ovary > brain. The enzyme activity is not consistent with the MPG mRNA level of our results. However, in contrast to MPG activity, the level of mRNA expression is higher in the newborn mice than in the young adults. Finally, whether the tissue-specific variation in MPG expression is a reflection of the transcription rates of the gene or results from altered stability of the mRNAs is not known.

REFERENCES

Barrow, L. R. and Magee, P. N. (1982) Nonenzymatic methylation of DNA by *S*-adenosylmethionine *in vitro*. *Carcinogenesis* 3: 349-351

Bellec, G., Dreano, Y., Lozach, P., Menez, J. F. and Berthou, F.

sodialkylamines by human liver microsomes. *Carcinogenesis* 17: 2029-2034

Bjelland, S. and Seeberg, E. (1987) Purification and characterization of 3-methyladenine DNA glycosylase I from *Escherichia coli*. *Nucleic Acid Res.* 15: 2787-2801

Bond, S. L. and Singh, S. M. (1987) Methyl nitrosourea induced DNA synthesis *in vivo* in mice: effects of background genotype on excision repair during aging. *Mech. Ageing Dev.* 41: 177-187

Chakravarti, D., Ibeanu, G. C., Tano, K. and Mitra, S. (1991) Cloning and expression in *Escherichia coli* of a human cDNA encoding the DNA repair protein N-methylpurine DNA glycosylase. *J. Biol. Chem.* 266: 15710-15715

Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162: 156-159

Craddock, V. M. and Henderson, A. R. (1982) The activity of 3-methyladenine DNA glycosylase in animal tissues in relation to carcinogenesis. *Carcinogenesis* 3: 747-750

Engelward, B. P., Boosalis, M. S., Chen, B. J. Deng, Z., Siciliano, M. J and Samson, L. D. (1993) Cloning and characterization of a 3-methyladenine/ 7-methylguanine/3-methylguanine DNA glycosylase cDNA whose gene maps to chromosome 11. *Carcinogenesis* 14: 175-181

Gallangher, P. E. and Brent, T. P. (1984) Further purification and characterization of human 3-methyladenine-DNA glycosylase: evidence for broad specificity. *Biochim. Biophys. Acta* 782: 394-401

Generoso, W. M. (1982) A possible mechanism for chemical induction of chromosomal aberrations in male meiotic and postmeiotic germ cells of mice. *Cytogenet. Cell Genet.* 33: 74-80

Gensler, H. I. and Bernstein, H. (1981) DNA damage as the primary cause of aging. *Quant. Rev. Biol.* 56: 279-303

Helland, D. E., Male, R., Haukanes, B. L., Olsen, L., Haugan, I. and Kleppe, K. (1987) Properties and mechanism of action of eukaryotic 3-methyladenine-DNA glycosylases. *J. Cell Sci. suppl.* 6: 139-146

Kim, N. K., Lee, S. H., Chee, Y. H., Ko, J. J., Roy, R. and Cha K. Y. (1997) Tissue-specific and age-dependent expression of DNA repair gene, N-methylpurine-DNA glycosylase in Balb/c mice. The 88th *American Association for Cancer Research in San Diego.* # 3743

Kovacs, E., Weber, W. and Muller, H. J. (1984) Age-related variation in the DNA repair synthesis after UV-c irradiation in unstimulated lymphocytes of healthy blood donors. *Mutat. Res.* 31: 231-237

Licastro, F. and Walford, R. L. (1986) Modulatory effect of nicotinamide on unscheduled DNA synthesis in lymphocytes from young and old mice. *Mech. Aging Dev.* 35: 123-131

Lim, I. K., Vician, L. and Herschman, H. R. (1994) Ligands induced PC12 cell- specific subtraction library. *Korean J. Biochem.* 26: 21-28

Lindahl, T. (1982) DNA repair enzymes. *Annu. Rev. Biochem.* 51: 61-87

Lindahl, T., Sedgwick, B., Sekiguchi, M. and Nakabeppu, Y. (1988) Regulation and expression of the adaptive response to alkylating agents. *Annu. Rev. Biochem.* 57: 133-157

Loeb, L. A. and Preston, B. D. (1986) Mutagenesis by apurinic/apyrimidinic sites. *Annu. Rev. Genet.* 20: 201-230

McCarthy, T. V., Karran, P. and Lindahl, T. (1984) Inducible repair of O-alkylated DNA pyrimidines in *Escherichia coli*. *EMBO J.* 3: 545-550

Mitra, S. and Kaina, B. (1993) Regulation of repair of alkylation damage in mammalian genomes. *Prog. Nucleic Acids Res. Mol. Biol.* 44: 109-142

Mitra, S., Roy, R., Tano, K., Ibeanu, G. C. Dunn, W. C. Kim, N. K. Natarajan, A. T., Hartenstein, B. and Kaina, B. (1992) Alkylation damage repair in mammalian genomes. In *DNA repair mechanisms* (Bohr, Vilheim, A. and Wasserman, Karsten, Kramer and Kenneth, H., eds.), Munksgaad, Copenhagen, Alfred Benzon Symposium 35: 328-339

Mullaat, E., Lohman, P. H. M., Berends, F. and Viig, J. (1990) DNA damage metabolism and aging. *Mutat. Res.* 237: 189-210

Nebert, D. W. and Gonzalez, F. J. (1987) P450 genes: strucrure, evolution, and regulation. *Annu. Rev. Biochem.* 56: 945-993

Park, J. W. and Ames, B. N. (1988) 7-Methylguanine adducts in DNA are normally present at high levels and increase on ageing: Analysis by HPLC with electrochemical detection. *Proc. Natl. Acad. Sci.* 85: 7467-7470

Pegg, A. E. (1984) Methylation of the O^6-position of guanine in DNA is the most likely initiating event in carcinogenesis by methylating agents. *Cancer Invest.* 2: 223-231

Roy, G., Roy, R. and Mitra, S. (1997) Quantitative reverse transcriptase polymerase chain reaction for measuring the N-methylpurine-DNA glycosylase mRNA level in rodent cells. *Anal. Biochem.* 246: 45-51

Samson, L., Derfler, B., Boosalis, M. and Call, K. (1991) Cloning and characterization of a 3-methyladenine DNA glycosylase cDNA from human cells whose gene maps to chromosome 16. *Proc. Natl. Acad. Sci.* 88: 9127-9131

Tatsuka, M., Ibeanu, G. C., Izumi, T., Narayan, S., Ramana, C. V., Kim, N. K., Roy, G. and Mitra, S. (1995) Structural organization of the mouse DNA repair gene, N-methylpurine-DNA glycosylase. *DNA and Cell Biology* 14: 37-45

Washington, W. J., Dunn, W. C., Generoso, W. M. and Mitra, S. (1988) Tissue- specific variation in repair activity for 3-methyladenine in DNA in two stocks of mice. *Mutat. Res.* 207: 165-169

Washington, W. J., Foote, R. S., Dunn, W. C., Generoso, W. M. and Mitra, S. (1989) Age-dependent modulation of tissue-specific repair activity for 3-methyladenine and O^6-methylguanine in DNA in inbred mice. *Mech. Ageing Dev.* 48: 43-52

Weeda, G., Ma, L., van Ham, R. C. A., Bootsma, D., van der Eb, A. J. and Hoeijmakers, J. H. J. (1991). Characterization of the mouse homolog of the XPBC/ERCC-3 gene implicated in xeroderma pigmentosum and Cockayne's syndrome. *Carcinogenesis* 12: 2361-2368

Wei, Q., Matanoski, G. M., Farmer, E. R. Hedayat, M. A. and Grossman, L. (1993) DNA repair and aging in basal cell carcinoma: a molecular epidermiology study. *Proc. Natl. Acad. Sci.* 90: 1614-1618