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2-Am,4,6-DNT Causes Genotoxicity of P53 Gene in NG108 Neuroblastoma Cell Lines

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ABSTRACT The 2-Amino,4,6-dinitrotoluene(2Am-DNT) is a non-enzymatic chemical metabolite of TNT (trinitro toluene) which is an important hazardous environmental pollutant. Genomic DNA was isolated from the NG108 cells treated with 2 Am-DNT for seven hours and also from untreated cells. DNA spanning p53 tumor suppressor gene exons 6-9 and parts of exon 5 and 10 was PCR amplified from both the control and treated genomic DNA's. Amplified PCR products from control and treated cells were of the same size using specific primers. Preliminary analysis of sequences of PCR products from treated cells and untreated cells showed several nucleotide changes indicating a possible genotoxicity role of 2 Am-DNT.

INTRODUCTION

Various human tumors have been demonstrated to be associated with the loss of heterozygosity of a 17 chromosome fragment bearing p53 gene (Jones and Nakamura 1992). These tumors include colon, breast, liver, lung, stomach, bone, bladder, and oesophagial tumors. Various carcinogens, like 2-amino-3-methylimidazo (4,5-f) quinoline (Iq), have been reported to induce position specific mutation in the p53 gene (Makino et al., 1992). p53 mutations in a series of cell lines derived from methylchlolanthrene induced mouse fibrosarcomas have been reported (Halevy et al. 1990).

These findings prompted us to analyze the effects of 2Am-DNT, which is a spontaneous non-enzymatic metabolite of the explosive TNT, and an environmental pollutant. The NG 108 neuroblastoma cell line was selected because p53 has the wild type form in this cell line at both loci (Castrasena et al. 1994). 2Am-DNT is the most common metabolic byproduct of Trinitrotoluene (TNT) which has been extensively used by the US Army and in the industry; it is found in soil and water. Various studies have shown toxicity of TNT and its metabolic byproducts (Rickert et al. 1983), however, to the best of our knowledge the effects of 2 Am-DNT on the p53 gene is not known.

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MATERIALS AND METHODS

NG108 neuroblastoma cells were originally donated by Dr. M. Nirenberg of the NIH. Culturing and maintenance of this cell line has been discussed previously (Dutta et al.1992). The cells were cultured in DMEM medium with 10% fetal calf serum at 37°C in a carbon dioxide incubator. Cells were exposed to 100 ppm of 2Am-DNT for 7 hours. Genomic DNA was isolated from control and treated cells following protocols of the genomic DNA isolation kit (Sigma Chemicals, USA).P53 gene exons 6-9 and part of exons 5 and 10 were PCR amplified using the primers 5' TGG CCC TCC TCA GCA TCT TA 3' and 5' CAA GGC CTC ATT CAG CTC 3'. PCR conditions were with the following profile: 94°C for 15 seconds, 58°C for 30 seconds, 72°C for 2 seconds followed by a final extension of 6 minutes at 72°C. The PCR products were then purified and sequenced by an automated DNA sequencer following standard protocol (Hou and Dutta 2000).

RESULTS

Tumor suppressor gene p53 sequences were amplified by PCR and de novo sequenced in both the forward and reverse directions. A 600 base pair PCR product was obtained. Figure 1 shows the same size PCR product using the normal p53 gene and NG108 cells, and also treated

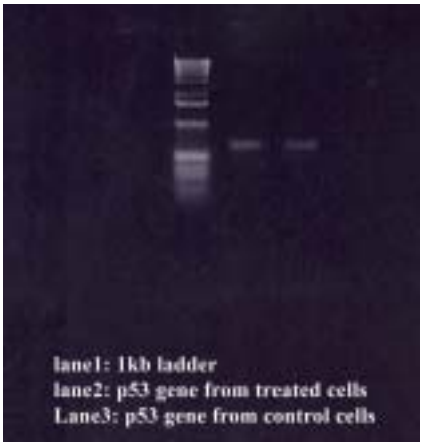

Fig. 1. p53 gene hot spot region amplified by PCR.
Lane 1: 1kb ladder
Lane 2: p53 gene product from control NG108 cells showing amplified p53 gene
Lane 3: p53 gene product from 2, 4 Am-DNT treated NG108 cells.

cells. That the forward and reverse sequences have homology was examined by comparing corresponding bases in the forward and reverse directions. The finding that 2Am-DNT causes p53 mutation suggests that it is a potential carcinogen, since p53 is considered the dosimeter for molecular carcinogenesis.

DISCUSSION

Our aim was to find out if 2Am-DNT is genotoxic by testing its mutagenic capability using the well konwn tumor suppressor p53 gene. Harris (1993) reported several p53 point mutations in liver cells induced by aflatoxin B1 which is a potential hepatocellular carcinogen. The p53 gene in its wild type form is a tumor suppressor gene whereas in its mutated form it is oncogenic promoting tumor growth (Harris 1993). We have documented, for the first time, the potential genotoxic activity of 2Am-DNT based on the sequencing of PCR amplified specific exons of

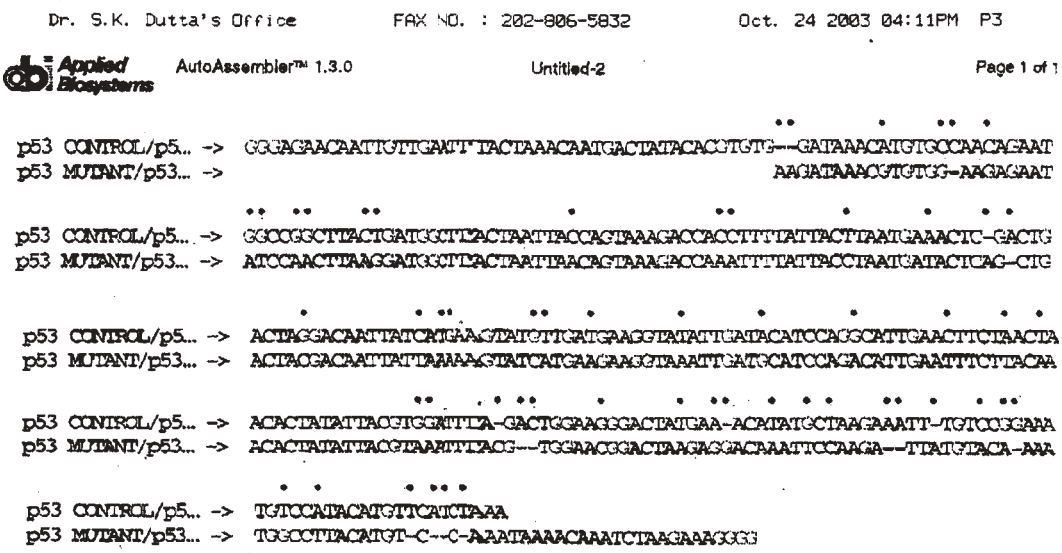

Fig. 2. Comparison of the reverse sequences by using EXON 10 reverse primer of the p53 gene obtained from the control and the 2Am-DNT treated cells.p53 wild type or control assemblage shows the different positions or numbers of the nucleotides. The dot marks compare the mutations or base changes at the particular position or number of the nucleotide. In this figure we are seeing several dots or base changes between the control and the treated p53 sequences, for example at nucleotide number 159, a T is in the control sequence whereas in the same position in the treated sequence there is an A, showing there is a transverse point mutation at that position. However, at some places the computer program has detected and put a dot mark for mutation when a base is completely missing which is due to faulty arrangement and reading of the base by the computer. Genbank accession numbers for these sequences are U92576 and U92577.

the p53 gene. Exon 6-9 and parts of exon 5 and 10 of the p53 gene were amplified using two primers spanning these region (Sjorgen et al. 1995). The amplified PCR products were then bidirectionally de novo sequenced by Applied Biosystem, USA, automated DNA sequencing apparatus (GenBank accessiohn # U92576 and U 92577). In both directions, single base pair substitution was found when the control cells p53 sequence was compared to that of the 2Am-DNT treated cells. p53 gene mutations are clustered in four 'hot spots' which exactly coincide with the four most highly conserved regions of the gene (Nigro et al. 1989).The outcome of the investigation presented here has implication in understanding the mechanism of chemical carcinogenesis.

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REFERENCES

Banarjee HN, Verma M, Hou LH, Ashraf M, Dutta SK 1999.Cytotoxicity of TNT and its metabolites.*Yale Journal of Biology and Medicine,* **72:** 1-4.

Castrasena JS, Bello MJ, Rey JA, Pestana A 1994. No TP53 mutations in neuroblastoma detected by PCR-SSCP analysis. *Genes, Chromosome and Cancer*, **10(2):** 136-138.

Das K, Ghosh B, Blackman CF1992. Dose dependence of acetylcholineesterase activity in neuroblastoma cells exposed to modulated radio-frequency elertromagnetic radiation. *Bioelctromagnetics*, **13:** 317-322.

Halevy O, Michalovitz D, Oven M 1990. Different tumor derived p53 mutations exhibit distinct biological activities. *Science,* **250 (4977):** 113-116.

Harris CC 1993. p53: At the cross roads of molecular carcinogenesis and risk assessment. *Science,* **262:** 1080-1081.

Hou L, Dutta SK 2000. Phylogenetic characterization of several para- and meta-PCB dechlorinating Clostridium species: 16s rDNA sequence analyses. *Lett Appl Microbiol,* **30(3):** 238-43.

Jones MH, Nakamura Y 1992. Detection of loss of heterozygosity at the human TP53 locus using a dinucleotide repeat polymorphism. *Genes, Chromosome and Cancer,* **5:** 89-90.

Miakino H, Ishizaka Y, Tsujimoto A, Nakamura T, Onda M, Sugumara T, Nago M 1992. Rat p53 gene mutations in primary zymbal gland tumors induced by 2-amino-3mthyl-imidzo[4,5-f]quinoline, a food mutagen. *Proc Natl Acad Sci USA,* **89(11):** 48500-48504.

Nigro, J.M., Baker, J.S., Preisinger, A.C., Jessup, J.M., Hostetter, R., Clearly, K., Blgner, S.H., Davidson N, Baylin S, Devilee P, Glover T, Collins FS, Weston A, Modali R, Harvis CC, Vogelstein B 1989. Mutations in the p53 gene occur in diverse human tunmor types. *Nature,* **342:** 705-708.

Rickert DE, Byron EB, James AP 1983. Dinitrotoluene: acute toxicity, oncogenicity, genotoxicity, and metabolism. *CRC Critical Reviews in Toxicology,* **13(3):** 217-233.

Sjogren, S, Inganas M, Torbjorn N, Lindgren A, Nordgren H, Holmberg L, Berg J 1995. The p53 gene in breast cancer: Prognostic value of complementary DNA sequencing versus immunohistochemistry. *J Natl Cancer Inst,* **88(3/4):** 173-182.