in vivo *14:* 437-440 (2000)

Deuterium Depletion can Decrease the Expression of C-myc Ha-ras and p53 Gene in Carcinogen-Treated Mice

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Abstract. *In spite of the fact that the deuterium concentration is over 10 mmol/l in all living organisms, its possible role has been ignored for six decades. Recent studies have shown that the depletion of the naturally occurring deuterium can result in tumour regression in mice, dogs, cats and humans. The effect of deuterium depletion on gene expression plays a key part in tumour development. The carcinogen, 7,12-dimethylbenz(α)anthracene (DMBA), was used to increase gene expression in "short term" investigations. The expression of c-myc, Ha-ras and p53 gene was followed in CBA/Ca sensitive inbred mice drinking tap water or deuterium-depleted water (DDW) after induction. By detecting the RNA expression 48 hours after exposure to the carcinogen it was found that the expression of all genes investigated was inhibited in six different organs (spleen, lung, thymus, kidney, liver and lymph node) in the DDW-treated group. It is suggested that genes playing a key role in the cell cycle regulation and tumour development are sensitive to deuterium depletion.*

It has been known for decades that the mass difference between hydrogen and deuterium leads to differences in the physical and chemical behaviour of the two stable isotopes (1-2). The effect of the replacement of hydrogen with deuterium (*e.g.* by the application of heavy water, D_2O) is also well documented (3-5). The possible role of naturally occurring deuterium, whose concentration is over 16 mmoi/l in surface water and 12-14 mmol/l in living organisms (6), in biological systems was first investigated in the early nineties. The results showed that the partial replacement of the naturally occurring deuterium with hydrogen by the application of deuterium-depleted water (DDW) inhibited cell growth rate (L_{929} fibroblast cell line) and caused complete tumour regression in xenotransplanted mice (7). Subsequent experiments revealed that deuterium depletion inhibited the proliferation of tumorous cell lines *in vitro* (PC-3 human prostate, MDA-MB-231, MCF-7 human breast and M14 human melanoma), caused regression of breast adenocarcinoma in dogs and showed efficacy in Phase II double blind clinical trial with human prostate cancer (8). The application of DDW also triggered apoptosis *in vitro* (8) and *in vivo* (9).

The *in vivo* detection of the expression of certain oncogenes and tumour suppressor genes in the "short term" experiment is a good model for the early detection of exposure to carcinogens (10-28). In order to investigate the possible influence of deuterium depletion on the expression of genes involved in malignant transformation, 7,12-dimethyl benz(α)anthracene (DMBA) was used to increase the expression of (proto)oncogenes and tumour suppressor gene in sensitive (10) inbred mice.

The present study was designed to examine whether deuterium depletion influence the expression of the c-myc, Ha-ras and p53 genes.

Abbreviations: DMBA, 7,12-dimethylbenz(a)anthracene, DDW, deuterium-depleted water.

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Key Words: Deuterium, deuterium depletion, deuterium-depleted water, DMBA, c-myc, Ha-ras, p53, gene expression.

Materials and Methods

Animals. 6 to 8-week-old CBA/Ca (Lati, Gödölló, Hungary) inbred mice were kept in a conventional breeding house.

Treatment. Each group was treated with a single dose of 40 mg/bwkg 7,12-dimethylbenz(α)anthracene, which was dissolved in corn oil and given intraperitoneally. The drinking water of the animals in the treated group (24 mice) was replaced for two days with DDW (21 ppm ± 3 ppm deuterium) after the injection of DMBA. The animals (12 mice) in the control group consumed tap-water (150 ppm ± 3 ppm).

Gene expression. 48 hours after the gene induction with DMBA and the subsequent treatment with DDW the animals were autopsied. For RNA isolation the lung, liver, kidney, thymus, spleen and lymph nodes were removed and pooled into two-two groups. The RNA was isolated with Trisol (GIBCO, Grand Island, NY, USA) according to manufacturer's instructions (29). RNA samples after OD control (A260/A280>1.8) were diluted to 0.1 μg/μl and blotted 100 μl onto Hybond N+ membrane (Amersham, Little Chalfont, UK) so that each blot contained 10 μg of total RNA. After heat fixation the membranes were hybridized with chemiluminescently labeled cloned DNA probes of c-myc, Ha-ras and p53 (American Tissue Culture Collection Manassas, USA). The hybridization protocol was carried out according to the manufacturers' instructions (ECL gene detection system, Amersham) (30). The signals

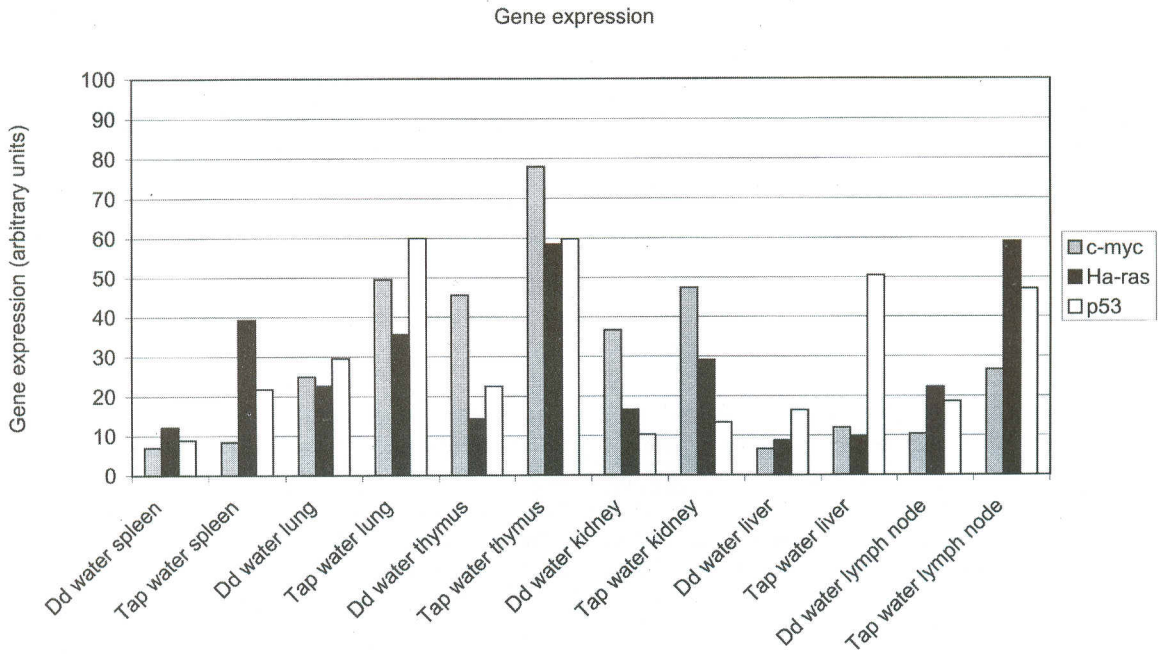

Figure 1. *Deuterium depletion inhibited, DMBA induced gene expression. Ha-ras, c-myc and p53 mRNA level were measured 48 hours after the DMBA inductuon in different organs (spleen, lung, thymus, kidney, liver and lymph node). The CBA/Ca mice received tap water (150±3 ppm deuterium) in the control group and deuterium depleted water (21±3 ppm deuterium) in the treated group for 48 hours.*

were detected on X-ray film, developed, scanned into the computer and analysed by Quantiscan software (Biosoft, Cambridge, UK). The level of c-myc, Ha-ras and p53 gene expression was determined by percentage of positive control.

Results and Discussion

It is known that, after carcinogen exposure of sensitive mice, an increase of certain (proto)oncogene and tumour suppressor gene expressions can be observed. The present study was meant to answer the question whether deuterium depletion can influence the early response of these genes, which have a central role in tumorigenesis. In order to cause deuterium depletion in mice, the drinking water (150 ppm ± 3 ppm) of the treated animals was replaced with deuterium depleted water (21 ppm ± 3 ppm). The effect of deuterium depletion on c-myc, Ha-ras and p53 gene expression was studied after inducing the expression of these genes with DMBA. The rate of gene expression of these genes was determined in six different organs (spleen, lung, thymus, kidney, liver and lymph node) of the mice. The results summarized in Figure 1, show that independently of the organs or the genes, the expression was lower in the DDW-treated than in the control group.

The RNA expression pattem in the investigated organs was different. Further investigations are required to reveal a possible tissue specificity. The results of the present study, however, clearly indicate the inhibitory effect of deuterium depletion on the expression of these genes.

The molecular pathomechanism of carcinogenesis involves the changes of expression of oncogenes and tumor suppressor genes (31). Decreasing of (proto)oncogene and suppressor gene (over)expression may decrease the risk of malignant transformation (32-35). Since our earlier study (7-9) proved that deuterium depletion can result in tumour regression in mice, dogs, cats and in humans, we assume that the genes that have key role in tumorigenesis are sensitive to a shortage of deuterium.

Several studies have proved the effect of deuterium at high concentration which is the consequence of isotopic effect. Heavy water was found to accelerate the rate of actin polymerization and to increase microfilament gelatlon (36), to arrest cell proliferation (37) and also to inhibit heat shock protein induction (38).

We would like to emphasize that the application of DDW results in a decrease of deuterium concentration close to the natural level. The present results also seem to support the hypothesis that naturally occurring deuterlum may be involved in the regulation of genes which have a key role in cell cycle regulation and/or in tumour development.

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Received December 3, 1999
Accepted February 23, 2000