

# The Biological Effects of Deuterium-Depleted Water, a Possible New Tool in Cancer Therapy

G. Somlyai<sup>1</sup>, G. Laskay<sup>2</sup>, T. Berkényi<sup>3</sup>, Z. Galbács<sup>4</sup>, G. Galbács,  
S.A. Kiss<sup>5</sup>, Gy. Jákli<sup>6</sup>, G. Jancsó<sup>6</sup>

## Zusammenfassung

Es ist bekannt, daß der Massenunterschied zwischen Wasserstoff und Deuterium zu Unterschieden im physikalischen und chemischen Verhalten zwischen den beiden stabilen Isotopen führt. Trotz der Tatsache, daß die Konzentration von Deuterium ungefähr 150 ppm (mehr als 16 mM) in Oberflächenwasser und mehr als 10 mM in lebenden Organismen beträgt, ist die mögliche Rolle, die natürlich vorkommendes Deuterium in biologischen Systemen spielt, vor 1993 nie untersucht worden. Die ersten Experimente mit Deuterium-abgereichertem Wasser (DDW) zeigten, daß aufgrund der Deuteriumabreicherung die nicht-tumorösen Fibroblastenzellen L<sub>929</sub> mehr Zeit benötigten, um sich in vitro zu vervielfältigen, und DDW verursachte eine Regression des menschlichen Brusttumors in Mäusen.

Im vorliegenden Artikel legen wir zusätzliche Beweise vor, die zeigen, daß DDW i.) die Zellwucherung der A4-Zell-Linie hemmt, ii.) als Trinkwasser das Wachstum des menschlichen Prostatatumors PC-3 in Mäusen hemmt und iii.) eine vollständige oder teilweise Tumorregression in Hunden und Katzen bei unterschiedlichen Tumoren induzieren kann.

Auf der Grundlage der mit DDW gemachten Beobachtungen nehmen wir an, daß die Zellen in der Lage sind, das Verhältnis von Deuterium und Wasserstoff zu regulieren und daß die Veränderungen im Verhältnis von Deuterium und Wasserstoff bestimmte molekulare Mechanismen auslösen können. Wir vermuten, daß die Anwendung von DDW neue Möglichkeiten bei der Krebstherapie eröffnen kann, indem ein direkter Eingriff in den Mechanismus ermöglicht wird, der eine zentrale Rolle bei der Regulierung des Zellzyklus spielt.

## Schlüsselwörter

Deuterium-abgereichertes Wasser, DDW, Krebs, Tumorregression, Zellsignalisierung, Krebstherapie.

## Summary

It is known that the mass difference between hydrogen and deuterium leads to differences in the physical and chemical behaviour between the two stable isotopes. In spite of the fact that the concentration of D is about 150 ppm (over 16 mM) in surface water and more than 10 mM in living organisms the possible role of the naturally occurring deuterium in biological systems was never investigated before 1993. The first experiments with deuterium-depleted water (DDW) revealed that due to the D-depletion the non-tumorous L<sub>929</sub> fibroblast cells required longer time to multiply in vitro and DDW caused human breast tumor regression in mice.

In this communication we present additional evidence demonstrating that DDW i) inhibits cell proliferation of A4 cell line in vitro; ii) as drinking water inhibits the PC-3 human prostate tumor growth in mice, induces apoptosis in vivo, iii) can induce complete or partial tumor regression in dogs and cats with different tumors.

Based upon the observations gained with DDW we suppose that the cells are able to regulate the D/H ratio and the changes in the D/H ratio can trigger certain molecular mechanisms. We suggest that the application of DDW may open new possibilities in cancer therapy by offering a direct intervention into the mechanism playing a central role in cell cycle regulation.

## Keywords

deuterium-depleted water, DDW, cancer, tumor regression, cell signaling, cancer therapy.

G. Somlyai<sup>1</sup>, HYD Ltd. for Research and Development, H-1539 Budapest, 114, P.O.B. 695; G. Laskay<sup>2</sup>, József Attila University, Department of Botany, H-6720 Szeged, Egyetem u. 2., T. Berkényi<sup>3</sup>, Alpha-Vet Veterinary Hospital, H-8000 Székesfehérvár, Homokosor u. 7., Z. Galbács<sup>4</sup>, G. Galbács<sup>4</sup>, József Attila University, Department of Inorganic and Analytical Chemistry, H-6720 Szeged, Dóm tér 7., S.A. Kiss<sup>5</sup>, Hungarian Magnesium Society, H-6726, Szeged, Fő fasor 73 A/2., Gy. Jákli<sup>6</sup>, G. Jancsó<sup>6</sup>, Atomic Energy Research Institute, H-1525 Budapest, P.O.B. 49.



## Introduction

It has been known for decades, that due to the mass difference between hydrogen (H) and deuterium (D), the behaviour of the molecules containing deuterium is altered in chemical reactions [1, 2]. The difference between hydrogen and deuterium manifests itself in biological systems, too. Heavy water with high deuterium concentrations has been widely used and the experience shows that it exerts a pronounced influence on the processes taking place in a given biological system [3, 4]. The significant effect of heavy water on the living organism is not a surprise, considering that an important part of the living body consists of water, and heavy water differs from ordinary water in many of its properties [5].

In spite of the fact that the concentration of D is about 150 ppm (over 16 mM) in surface water and more than 10 mM in living organisms, the presence of the naturally occurring deuterium (NOD) has been practically ignored. In order to investigate whether NOD has any role in living organisms we applied deuterium-depleted water (DDW) to prepare medium for tissue culture and also as drinking water to treat mice xenotransplanted with human breast tumor [6]. The results revealed that due to the D-depletion the non-tumorous L<sub>929</sub> fibroblast cells required longer time to multiply in vitro and DDW caused tumor regression in mice. Our recent results [7] suggest that NOD may have a central role in the regulation of the intracellular processes of different biological systems.

In order to reveal the potential usefulness of DDW in cancer therapy the double blind phase II clinical was launched with prostate cancer patients in 1995. The interim evaluation of the clinical trial showed a significant effect.

In this communication we present further evidences that the D depletion caused by the replacement of normal water with DDW can inhibit tumor growth.

## Results

*DDW-media had an inhibitory effect on the initial growth rate of cells in tissue culture.*

To evaluate the effect of DDW the A4 cell line (A4: murine haemopoietic cell line FDCP-Mix, clone A4) was exposed to media prepared with DDW (90 ppm D) and with normal distilled water (150 ppm D) as control. Figure 1 shows that the D depletion inhibited the initial growth rate of A4 cells in culture in the first 10–12 hours. This confirmed our earlier findings [6], where L<sub>929</sub> mouse fibroblast cell line was tested, that in the culture fluid prepared with DDW the multiplication of the cells started with a delay of 5–10 hours, but thereafter the medium with DDW had only a minimal effect on the growing.

*DDW as drinking water induced tumor regression in mice with transplanted human tumors.*

In order to extend our investigation on the effect of DDW on tumor growth, human prostatic PC-3 cells were trans-

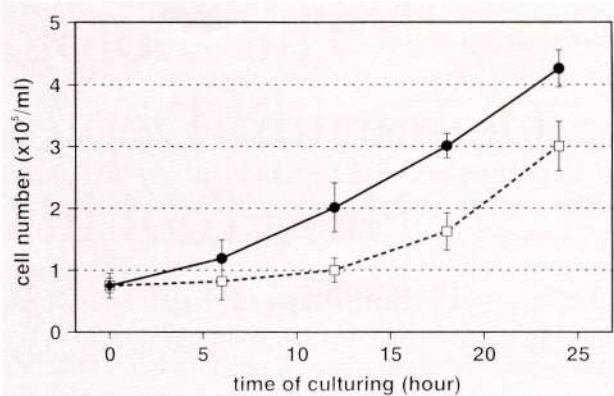


Fig. 1: The effect of deuterium-depleted water on the initial growth rate of A4 cells in culture (●: 150 ppm D, □: 90 ppm D)

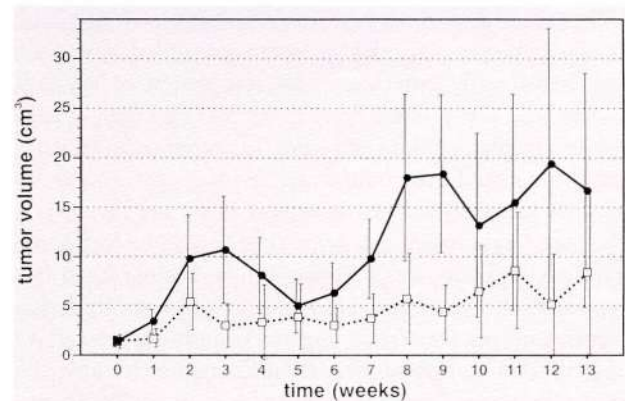


Fig. 2: The effect of deuterium-depleted water on the volume of PC-3 human prostate tumor in mice (●: 150 ppm D, □: 94 ppm D)

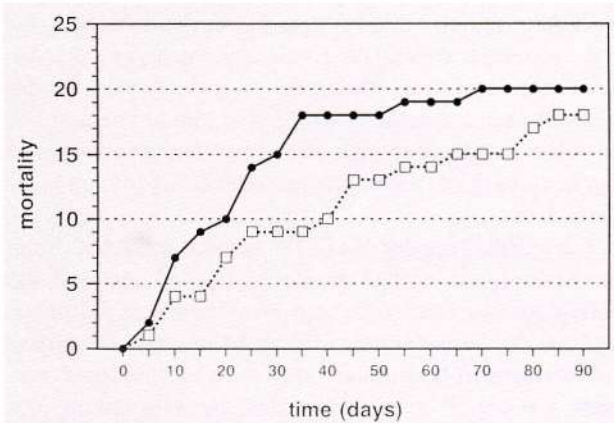


Fig. 3: Cumulative mortality of mice after transplantation with PC-3 human prostate tumor (●: 150 ppm D, □: 94 ppm D)

planted into 22–22 (control and treated) immunosuppressed CBA/Ca mice. The regular drinking water in the treated group was replaced by DDW (94 ppm D) on the 32nd day after the transplantation, which is a relatively late stage of tumor development. Tumor volume (Fig. 2) and the time of survival were monitored (Fig. 3). Although the average tumor volume was considerably smaller in the treated group but due to the high standard deviation the differences cannot be considered as being significant. The



time of survival was lengthened in the treated group by 40 % which also can be viewed as an evidence for the antiproliferative effect of DDW on cancer cells.

The inhibitory effect of DDW on tumor growth was more clearly revealed when the tumor growth was followed individually in the animals. In the control group the tumor size was gradually increasing in each animal, except in one. In the DDW-treated group the tumor volumes were found to increase continuously only in 8 out of 22 animals whereas partial tumor regression was found in 7 animals, after the treatment had started on the 32nd day. In four additional cases where the tumor volumes were only 0.1–0.2 cm<sup>3</sup> on the 32nd day, the tumor growth was almost completely inhibited for 2–4 weeks, while three animals exhibited complete tumor regression.

In the next experiments with PC-3 tumor cells, following the transplantation of the tumors into the mice, the normal water was replaced with DDW (98 ppm D) on the 18th day after transplantation in the treated group. 12 days later all the animals were killed, the tumors were removed and histologically examined.

A high growth rate of tumors was evident macroscopically in the control group, inasmuch as the average tumor size was 40 % larger than that in the DDW-treated group. The shape of the tumors was irregular, while in the treated group mice were bearing small, compact, almost regular ball-shaped tumors. The results revealed that in those animals which had received normal water, 3.6 % of the cells were in mitosis and 1 % were in apoptosis. The ratio was almost the opposite in the treated group, where only 1.5 % of the cells were in mitosis, while 3 % of the cells were in apoptosis. This result corroborates our earlier finding that DDW not only inhibits proliferation but may also trigger apoptosis [8].

#### *Consumption of DDW as drinking water leads to tumor regression in dogs and cats.*

First a nine-year-old, male cat was treated with DDW (95 ppm D). The cat had no appetite, had been coughing for three weeks, showed the symptoms of anaemia and was in very poor condition. Two of the lymphatic glands (1–1.5 cm in size) were removed and histologically characterized as being lymphoid leucosis.

In the first two weeks, after the start of DDW treatment, the initially lying, weak cat began to eat and became lively. It gained weight, its condition improved. After three weeks of treatment the cat became symptomless. At this time another lymphatic gland was removed which still showed the earlier diagnosis. The next affected lymphatic gland was investigated five months later and at that time the histological changes were remarkable because lymphatic hyperplasia was diagnosed. 35 months after the first diagnosis the cat was still alive and was in a good and stable condition.

Three other cats were treated with lymphoid leucosis and in each case significant improvement was observed after the treatment had been started.

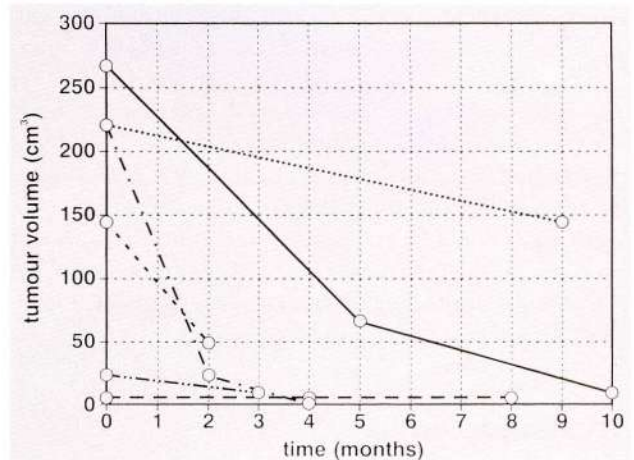


Fig. 4: The effect of deuterium-depleted water on breast carcinoma in dogs (No. 1 —, No. 2 ·····, No. 3: - - -, No. 4: - · - ·, No. 5: — · — ·, No. 6: —)

The first DDW treated dog had a mammary tumor. The size of her primary tumor was 271 cm<sup>3</sup> and in addition several smaller metastases of 2–3 cm diameter were palpable. The pathologists gave a histologic diagnosis of an adenocarcinoma. The consumption of the DDW (95 ppm D) led to the complete regression of the smaller metastases which was accompanied by the diminishing volume of the primary tumor. After five months the size of the tumor was 66 cm<sup>3</sup>, and after further four months 8 cm<sup>3</sup>, when we removed it surgically. The dog has been free of any symptoms since the last operation.

The responses of six dogs with breast tumor to the DDW-treatment are summarized in Fig. 4, which shows that there was a rapid response in two cases (No. 1, 3), in three dogs (No. 2, 4, 5) we were able to achieve a 35–67 % decrease in tumor size within 2–9 months. In one case (No. 6) the tumor size had not changed for 8 months, but the number of metastasis decreased.

The use of the DDW offers a possibility to treat the tumorous diseases in the field of the veterinary medicine. The applied daily dose of 0.01–0.02 kg DDW (95 ppm D) per kg of bodyweight seemed to be effective.

## Conclusions

During the past years we investigated the effect of decreasing the deuterium concentration of the medium of tissue culture, of the drinking water of mice, cats and dogs. We experienced that the decrease of the deuterium concentration resulted in significant changes proving that the life – having been adapted to a deuterium concentration of about 150 ppm during millions of years – perceives the deficiency of D. Taking into consideration the intense sensitivity experienced in the tumors at the withdrawal of deuterium, one can suppose that the adaptation is slower or absent in cancerous cells.

Our working hypothesis is that the cells are able to regulate the D/H ratio and that changes in the D/H ratio can trigger



certain molecular mechanisms having a key role in cell cycle regulation. We suppose that not the shift in intracellular pH [9–12] but the concomitant increase in D/H ratio is the real trigger for the cells to enter into S phase. The decrease of D concentration can intervene in the signal transduction pathways thus leading to tumor regression. We suggest that the application of DDW may open new possibilities in cancer therapy by offering a direct intervention into the processes of cell cycle regulation. The interim evaluation of the ongoing phase II double blind clinical trial with prostate tumor patients support our preclinical results.

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Correspondence address:

G. Somlyai, PhD

HYD Ltd. for Research and Development  
H-1539 Budapest 114, P.O.B. 695, Hungary  
Phone/Fax: 36-1-319-8976  
E-mail: gsomlyai@hungary.net



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