„I could never believe that the wonderful subtlety of biological reactions should be brought about by clumsy, relatively unreactive macromolecules without the concurrence of much smaller and more mobile units which could hardly be anything else than electrons."

Albert Szent-Györgyi: International Journal of Quantum Chemistry; Quantum Biology Symposium; The living state and cancer 7, 217-223 (1980)

DEUTERIUM HAS A KEY ROLE IN TUMOUR DEVELOPMENT – A NEW SUBMOLECULAR REGULATORY SYSTEM

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Introduction

The deuterium/hydrogen (D/H) mass ratio is the largest among stable isotopes of the same element, causing differences in the physical and chemical behaviour between the two hydrogen isotopes. Although the concentration of D is more than 10 mM (150 ppm) in living organisms, the potential role of D was not investigated for six decades.

Aim

In order to reveal the possible role of naturally occurring D in living organisms, the consequence of the shortage of D was investigated in different biological systems.

Material and Methods

To reduce the D-concentration in different biological systems below the natural level we used deuterium depleted water (DDW) in a range of 25 ppm and 135 ppm (D has a natural abundance in Earth's oceans of about one atom in 6,420 of hydrogen, ~156.25 ppm on an atom basis).

Results

The experiments with DDW revealed that due to D-depletion the cell growth of various cell lines (PC-3, MDA, HT-29, M14) were inhibited *in vitro*. Deuterium depletion also inhibited the expression of genes (c-myc, H-Ras, Bcl-2, K-Ras, COX-2) having key role in tumor development.

During the administration of DDW (85ppm) to prostate cancer patients in a phase II, double blind clinical trial, the net decrease in the prostate volume was three times higher in the treated group (160.3 cm^3 vs. 54.0 cm^3, $p=0.0019$). During the extended follow-up of the 44 patients, in the first year (from the date of entering the trial), 2 patients (9.1%) died in the treated group and 9 patients (40.9%) in the placebo group (significantly lower mortality in the treated group; Fisher's Exact Test, $p=0.034$).

Conclusion

We suggest that cells are able to regulate D/H ratio and its changes can trigger molecular mechanisms having key role in cell cycle regulation. The decrease in D-concentration can intervene into a hitherto unknown submolecular regulatory system which can serve as new target in anticancer drug development. This approach to D-depletion of water and other molecules has broad potential to enhance the effectiveness of the currently available oncotherapies and results in innovative new medicines.

The effect of D-concentration on the growth rate of different cancer cell lines *in vitro*

In order to get more insight into the mechanism of action of D-depletion, the inhibitory effect was studied using the real-time, label-free measurement of the xCELLigence RTCA system (Roche Applied Sciences). The System measures electrical impedance across Interdigitated micro-electrodes integrated on the bottom of tissue culture E-Plates. A dimensionless parameter called Cell Index (CI) is able to describe the viability of the cells and the cell count.

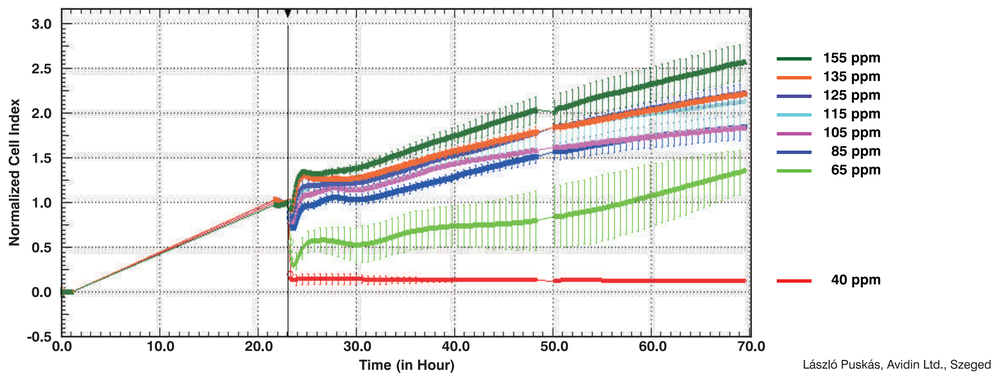

Figure 1. Real-time, label-free measurement of the inhibitory effect of different D-concentrations in A549 lung carcinoma cells using the xCELLigence RTCA system (Roche Applied Sciences)

Treatment with D-depleted media resulted in significant decrease in CI and the inhibitory effect was concentration-dependent. Complete inhibition was observed at 40 ppm D.

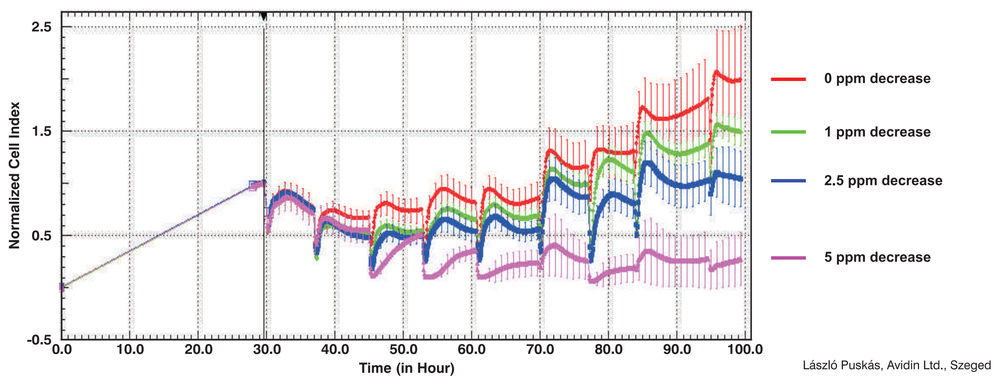

Figure 2. Real time, label free measurement of the effect of multi-step decrease of D-concentration by 1 ppm, 2.5 ppm or 5 ppm in the culture media 8 times in every 8 hour in A549 lung carcinoma cell line using the xCELLigence RTCA system (Roche Applied Sciences)

The CI index was already significantly lower as a result of the decrease of D by 1 ppm in comparison to control. After the 3rd and 4th decrease by 1 ppm, 2.5 ppm and 5 ppm the dose-dependency was detectable during the monitoring period of 90 hours.

DDW significantly decreased the growth rate of L929 fibroblast, HT-29 colon, A4 hematopoietic, MDA-MB-231 and MCF-7 breast, PC-3 prostate, HT-199 and M19 melanoma cell lines *in vitro*.

Effect of D-concentration on the early expression of c-myc, Ha-ras, Bcl-2, K-Ras oncogenes and p53 tumor suppressor gene in carcinogen-treated mice

The subgroups of eight-week old CBA/Ca mice were treated intraperitoneally (IP) with 7,12-dimethylbenz(a)anthracene (DMBA) and given DDW as drinking water (25 ppm D) or normal water (150 ppm D) *ad libitum.* Control groups underwent no carcinogen exposure, and were watered with DDW or normal water.

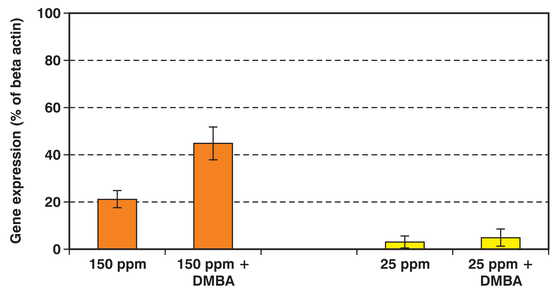

Figure 3.a Expression of Bcl-2 gene in lung tissue of CBA/Ca mice

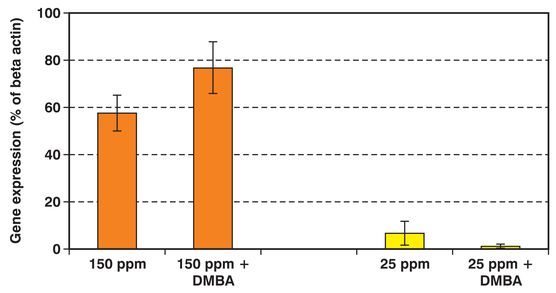

Figure 3.b Expression of K-ras gene in lung tissue of CBA/Ca mice

DMBA up-regulates Bcl2 and Kras proto-oncogenes in mice, and the expression of these genes was significantly lower after carcinogen exposure in case the animals were watered with DDW. The difference was significant between groups drinking DDW (25 ppm D) or normal water in DMBA-treated mice and also in untreated mice ($p<0.05$). The DMBA-induced expression of Ha-ras C-myc, p53 genes was also attenuated in CBA/Ca mice, when the water intake of the animals was replaced by DDW.

Deuterium depletion induces apoptosis *in vivo*

CBA/Ca mice were xenotransplanted with PC-3 tumor cells and on the 18th day after the intervention the water intake was replaced with DDW (98 ppm) in the treated group. Animals in the control group drank normal water (150 ppm D). Twelve days later the animals were killed, and the tumors were removed. The histological evaluation revealed, that DDW was able to reduce the proportion of mitotic cells, while a significantly higher proportion of tumor cells showed signs of apoptosis.

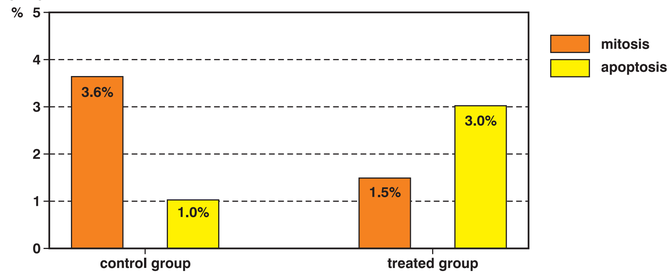

Figure 4. Effect of DDW on the viability of cancer cells in mice xenotransplanted with PC-3 human prostate tumor.

Clinical evidences

Phase II clinical trial

A double blind, four-month long, randomized human phase II clinical trial was conducted on 44 prostate cancer patients. The daily water intake was replaced with DDW (85 ppm D) in 22 patients (treated group), while the other 22 patients (placebo group) took normal water. Treatment was carried out in addition to the conventional forms of treatments.

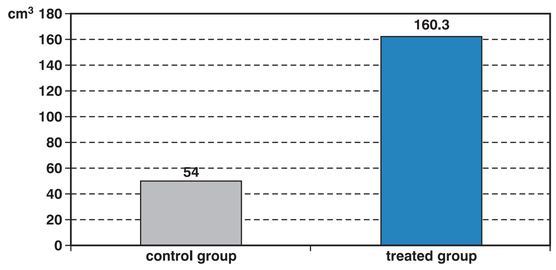

Figure 5. Cumulative net decrease in the prostate volume in the control and DDW-treated group of prostate cancer patients

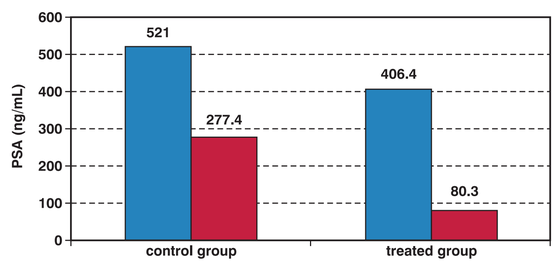

Figure 6. Cumulative change in the PSA values in the control and DDW-treated group of prostate cancer patients.

Both the net decrease in the prostate volume and the changes in PSA were significantly higher in those prostate cancer patients who underwent DDW-treatment in addition to the conventional therapies. The distribution of the best response (PR) to treatments differed significantly after four-month long DDW- or placebo treatment in the test groups (7subjects vs. 1 subject). All these results explain the impressive difference in the death rate in the two groups (2:9, treated vs. placebo group) within the first year ($p=0.034$).

Conclusion

We suggest that the naturally occuring D plays a key role in cell cycle regulation. Cells are able to regulate D/H ratio, and its changes can intervene into a hitherto unknown submolecular regulatory system (SMRS) in the signal transduction pathway. Cancer cells proved to be extremely sensitive to D-depletion, while non-cancer cells are able tolerate the decreasing D-concentration. D-depletion also inhibited the expression of the oncogenes in the different organs of carcinogen exposed mice. This approach to D-depletion of water and other molecules has broad potential to enhance the effectiveness of the currently available oncotherapies and results in innovative new medicines.

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