



Based on the factual knowledge gained by the research staff of HYD LLC on the biological effects and therapeutic effectiveness of deuterium depletion, a new type of pharmaceutical industry will develop in the near future.

# **Deuterium Depletion**

# New target for drug development

In early 90s, Gábor Somlyai, Hungarian molecular biologist, reported for the first time the inhibitory effect of decreased deuterium (the heavy isotope of hydrogen) concentration on the growth of different tumorous cell lines in vitro and in vivo [Somlyai et al., FEBS Letters (1993). 317, 1-4.]. The effect of gradual depletion of deuterium content of the culture media on tumor cell proliferation was investigated on ovarian and breast carcinoma. It was found that in these cell cultures DDW induced significant inhibition of tumor growth. Deuterium depletion repressed cell division in plants and suppressed certain genes, involved in tumour formation. The effect of deuterium-depleted water (DDW) on tumor growth was investigated in immunosuppressed mice transplanted with MDA-MB-231 and MCF-7 human breast and PC-3 prostate tumor. DDW halted or reversed tumor growth in vivo. In animal studies carried out with dogs and cats having different spontaneous tumors (breast, rectum, lymphoid leucosis, epithelioma, sarcomatoid tumors and melanoma), in spite of the various diagnoses, all tumors responded favourably to DDW treatment. The scientific results clearly show that a submolecular regulatory system exists in the cells, which is able to maintain homeostasis of the D/H ratio, thereby regulating gene expression and enzyme activity. Based on the differential handling of deuterium by sick vs healthy cells, deuterium depletion can serve as a new target for product development. HYD's approach to deuterium depletion of water and other molecules has broad potential to enhance the effectiveness of the presently available oncotherapies and results in innovative new medicines. Recent results shows that deuterium depletion - a novel tool of submolecular medicine - can address other indications such as diabetes. Results of prospective and retrospective human clinical studies supported the therapeutic effectiveness of DDW in tumorous and metabolic diseases, and in sports medicine.

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# Tumorous diseases

# **Prostate cancer**

In order to investigate the anticancer effect of DDW in humans a four-month long double-blind phase 2 placebo controlled clinical trial was conducted on prostate cancer (OGYI 5621/40/95). The primary outcome was the best response, and the agent's safety was also assessed. Forty-four patients were evaluated, 22 patients were involved in the treated-, and 22 patients in the placebo group, both groups received the same forms of conventional treatment. Summarizing the changes in prostate volume during the 4 months' period of the trial in the treated group a net decrease of 160.3 cm³ was achieved, on the contrary, the result was 54 cm³ in the control group. 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).

In addition, beside the 44 evaluated patients in the phase 2 clinical trial, the course of the disease was also retrospectively evaluated in 91 patients consuming DDW parallel with the conventional forms of treatment. 20 out of 91 retrospectively followed patients developed distant metastasis within one year after the diagnosis. The median survival time (MST) was 5.4 year, while the historical control is 1.2-1.6 years.

The results suggest that DDW might reduce the mortality of prostate cancer, since it was able to delay progression as well as to prolong MST in patients with histologically confirmed prostate cancer.

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## **Breast cancer**

The impact of DDW on breast cancer patients was evaluated in a retrospective study. Among the 232 patients, involved between February 1993 and April 2011, 158 had early stage breast tumor at the beginning of DDW consumption, and 74 had progressed stage tumor with distant metastasis. In the early stage breast cancer patients involved, median survival time was 217 months (18.1 years). In the subgroup where the patients started to drink DDW after the conventional therapy, in tumor-free state (in complete remission), extraordinarily good results could be achieved: median survival time was impossible to calculate because of the very low death rate during the follow-up period. In cases where the patient repeated DDW cure at least once, median survival was 293 months (24.4 years) which underlines that repeated lowering of deuterium level may play an important role also in prevention of relapse or metastasing. In a separate study, the effect of DDW on the survival in a patient group with distant metastatic breast cancer was analyzed. Data of 74 female patients between January 1993 and May 2005 were evaluated. All but 6 of the 74 patients had previously undergone intensive and repeated conventional therapy and their expected survival time at the beginning of the study was merely a few months. In the 74 evaluated patients 135 distant metastases were diagnosed before DDW treatment. DDW was applied in parallel with conventional cancer therapy in a supplementary manner, and the total daily water intake of the patients was covered by DDW. Simultaneous DDW and conventional treatment resulted in complete or partial reduction, or stagnation, of the tumor volume in 74.3% of the 74 patients evaluated. Median survival time from the diagnosis of the distant metastasis was 47.7 months, in contrast to 20-22 months found in literature sources. Likelihood of two-year survival of patients with distant metastases, consuming DDW simultaneously with conventional therapies, was 77.8%, while that of patients receiving only conventional therapies was 20%.

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# Lung cancer

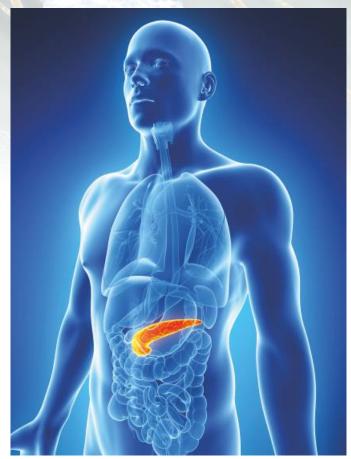
The effect of DDW on lung tumors and on expectable survival of lung tumor patients was evaluated in a retrospective study. From the data of the 129 patients involved, median survival increased for men from the expectable 7.5 to 25.9 months, and for women, from 11.3 to 74.1 months as a result of supplementary DDW application; while in the average for both sexes, median survival was 33.7 months. In cases of non-small cell lung cancer with brain metastasis, expectable median survival time is usually 19 to 27 months, but in our study it was 31.1 for both sexes. Application of DDW thus prolonged the expectable median survival time of lung cancer patients 2 to 4fold, compared to the patient population not consuming DDW. In certain cases, several years of survival or even complete remission was observed instead of the usually expectable few months. The cases of four lung cancer patients with brain metastasis who consumed DDW have been presented in our paper published in the Journal of Cancer Therapy.

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# Pancreatic cancer The effect of DDW on pancreatic tumors was evaluated in in vitro tests and a retrospective clinical study. In the in vitro tests, the

tests and a retrospective clinical study. In the in vitro tests, the effect of DDW was studied alone and in combination with the cytostatic Cisplatin on a Gemzar-resistant MIA PaCa-2 pancreas tumor cell line by means of the xCELLigence RTCA system (Roche Applied Sciences). This method is of advance because the cells are being monitored under physiological conditions, and cell division can be followed in real time without radioactive labelling and manipulating the cells. In the tests, changes of electric resistance are measured by means of a meshwork of microelectrodes on the bottom of the culture dish. The measured impedance increases in parallel with the growth and adhesion of the cells. This is described with the normalized cell index (CI). DDW (with 135, 125, 115, 105, 85, 65 and 40 ppm deuterium) inhibited the growth of MIA PaCa-2 pancreas tumor cells in vitro in a dose-dependent manner, with significant (p<0.02) CI decrease vs. control (150 ppm D). The cytotoxicity of Cisplatin was tested at the concentrations 20, 40 and 60 µM, combined with DDW (50 ppm D), on the MIA PaCa-2 cells. Combined application dose-dependently decreased CI, and synergism was observed. This raised the possibility that the same efficiency could be achieved with lower concentrations of the cytostatic, that is, with lower toxicity. Cisplatin showed maximum efficiency at 40 µM in presence of 50 ppm D, or already at 20 µM if 25 ppm deuterium was in the medium. This is another proof of synergism between Cisplatin and DDW in Gemzar-resistant MIA PaCa-2 cells. The results clearly show that combination of DDW and chemotherapy allows lowering the dose of cytostatics, with preserved efficiency but substantially reduced harmful side effects. In the retrospective clinical study, the survival of pancreas tumor patients was increased 6.5 times - from 6 to 39 months - by supplemental application of deuterium depletion, if DDW treatment was started within 60 days after diagnosis (n=18). In those patients (n=14) who were involved in the study more than 60 days after diagnosis, MST was 16 months.

DDW inhibited the growth of MIA PaCa-2 pancreas tumor cells in vitro. Applied together with conventional therapy, DDW prolonged MST of patients with progressive, inoperable pancreatic cancer 4-6 times.



**Reference:** Boros L.G., Meuillet E.J., Somlyai I., Jancsó G., Jákli G., Krempels K., Puskás L.G., Nagy L., Molnár M., Laderoute K.R., Thompson P.A., Somlyai G. (2014) Fumarate hydratase and deuterium depletion control oncogenesis via NADPH-dependent reductive synthesis. AACR 2014 - Annual Meeting, April 5-9, San Diego, CA, USA, DOI: 10.12918/HYD2014AACRPOST

# Metabolic diseases

### Effect of DDW on insulin resistance

The major characteristic feature of metabolic syndrome (MS) is the decreased insulin sensitivity. Insulin resistance/hyperinsulinemia is strongly associated with hyperlipidemia and hypertension, two major risk factors of coronary heart disease. In preclinical animal studies DDW enhanced insulin effect and potentiated glucose uptake in diabetic animals. Serum glucose, fructose amine, HbA1C values were significantly lower in those animals received DDW as drinking water and insulin treatment. Based on the experimental data, DDW could be used to treat patients with MS by increasing the insulin sensitivity.

In a human clinical investigation 30 volunteers with decreased glucose tolerance underwent 90 days long DDW treatment and physiological parameters characteristic to insulin resistance were evaluated to investigate the impact of DDW on these parameters. Evaluating the serum insulin concentration in the entire cohort relative to the values at day 0 during the intravenous glucose tolerance test (IVGTT), we found that serum insulin concentration decreased in 15 volunteers (day 0: 18.0±13.3  $\mu$ U/ml, day 90: 7.7±4.3  $\mu$ U/ml, p=0.007). In the group of patients with decreased insulin concentration significant (p=0.008), positive correlation was verified in the decrease of the serum glucose levels (day 0: 5.58 mmol/l, day 90: 5.24 mmol/l). Insulin resistance decreased in more than 30% of the volunteers, due to the increase of the glucose disposal from 6.9±2.4 mg/bwkg/min to 8.6±2.5 mg/bwkg/min. The fact, that DDW simultaneously effects insulin-, glucose values, and insulin resistance confirmed a strong correlation between these physiological parameters that describe the metabolic syndrome and it also suggests, that Dconcentration in the body might have an impact on these parameters.



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# Sports medicine

## Effect of DDW on the performance of athletes

In a sports medical test, the effect of DDW on the performance of top athletes was investigated, together with investigation of effects on metabolic processes, primarily glucose metabolism and acid-base balance, concomitant with physical load. Twelve international-level male rowers participated in the study, of who 7 athletes consumed 2 liters of deuterium-depleted drinking water (Preventa® 105) for 44 days, while 5 athletes drank ordinary tap water. Both groups had the same preparatory training. On day 0 and day 44, both groups underwent a multistage load test. They covered 4 x 1500 meters with increasing intensity, with 2-minute breaks between the stages. Pulse rate was continuously monitored. Acid-base parameters (pH, pCO<sub>2</sub>, pO<sub>2</sub>, HCO<sub>3</sub>, base excess), ions (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>), blood lactate and blood glucose, as well as the anion gap were determined from capillary blood samples at rest (R), in each stage under load (L1-L4), and in the 5th minute of restitution (R5'). The measurement results verified that in deuterium-depleted state achieved by consumption of DDW, tissue oxygenation improved, tissue anoxia was less pronounced, mobilization and utilization of glucose was better, and the cells showed better metabolic compensation of the loaddependent changes. It is well-known that, on physical load, first there is a decrease of blood glucose level due to the increased energy demand. This phenomenon was found in the control group at the beginning, at repeating the load test on day 44, and also in the DDW-treated group on day 0. In contrast to the above, however, no decrease of glucose level was observed in the treated group after consuming DDW for 44 days. In this group, the difference against the pre-treatment values was significant at load level L1 and L2. Before treatment, glucose level decreased by 25-34% at L1 and L2, but only by 5-7% after consumption of DDW. At load level L4, glucose level was elevated minimally (9% increase from resting level) in the first load test, but after DDW consumption this elevation was much higher - 46% increase from resting level. In absolute concentrations, the decrease at L1 and L2 was 1.9-2.6 mM/L, and the increase at L4 merely 0.4 mmol/L. After drinking DDW for 44 days, the initial glucose level



decrease was not more than 0.4-0.5 mmol/L, while increase in the second half of the load test was 2.5 mmol/L. After DDW consumption, blood lactate was significantly lower than in the first load test already in resting state, and the difference remained significant at load levels L1-L3. These results indicate that, after DDW consumption, the athletes performed the load stages with lower blood lactate levels, which possibly means that the cells became anoxic less or later on physical load, or lactate elimination was improved. The examination results were concordant with the athletes' subjective experiences, namely that after drinking DDW, they had improved performance, regenerated faster, and had better stamina on bodily load. DDW may thus be suitable for improving the performance of the organism in situations with increased physical load.

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