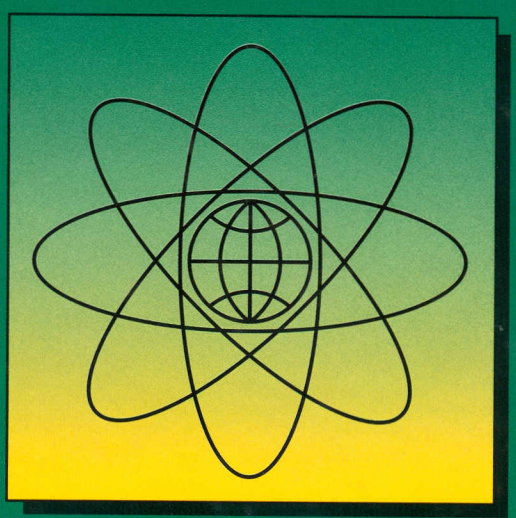
Synthesis and Applications of

ISOTOPICALLY LABELLED COMPOUNDS 1997



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Paper 24

NATURALLY OCCURING DEUTERIUM MAY HAVE A CENTRAL ROLE IN CELL SIGNALLING

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It is known that the deuterium/hydrogen (D/H) mass ratio is the largest of stable isotopes of the same element, causing differences in the physical and chemical behaviour between the two hydrogen isotopes (Wiberg 1955). The experiments carried out so far with D_2O in different biological systems (Rundel *et al.* 1988, Katz and Crespi 1971) and other experiments which revealed the importance of hydrogen bond (Fersht 1987) or intracellular pH in biological processes (Moolenaar *et al.*1983, Pouysségur *et al.*1984, Perona and Serrano 1988), have neglected the naturally occurring deuterium (NOD), 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.

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 tumour (Somlyai *et al.* 1993). The results revealed that due to the D-depletion the non-tumorous L_{929} fibroblast cells required longer time to multiply in vitro and DDW caused tumour regression in mice.

Our recent results suggest that the NOD may have a central role either in cell cycle regulation or in apoptosis.

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 (murine haemopoietic cell line FDCP-Mix, clone A4) were exposed to media prepared with DDW (90 ppm D) and with normal distilled water (150 ppm) as control. Fig. 1. shows that the D depletion inhibited the initial growth rate of A4 cells in culture in the first 10-12 hours.

Three tumorous cell lines (PC-3: human prostate, MCF-7: human breast, M14: human melanoma origin) also were exposed to DDW and the ^{3}H-thymidine incorporation was measured to determine the effect of deuterium depletion on DNA synthesis. There was an inhibition of ^{3}H-thymidine incorporation in the three non-synchronized cell lines which lasted for six hours in PC-3 and M14 cell lines ($p< 0.01$) and for 24 hours in MCF-7 ($p<0.03$) cell line. Calculating the ratio of proliferating cells on the basis of ^{3}H-thymidine incorporation in non-synchronized cells exposure to DDW-media for 1 hour

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caused 15%, 10% and 16% inhibition of cell proliferation in PC-3, MCF-7 and M14 cell lines, respectively. The inhibition was slightly higher when the cells were synchronized before DDW exposure.

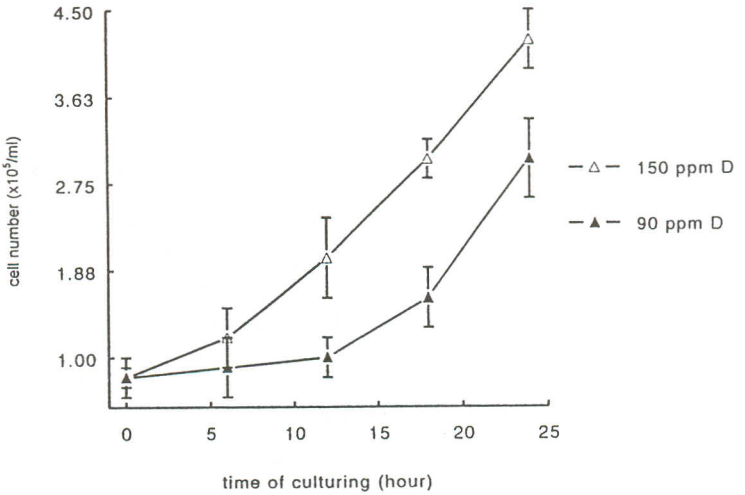

Fig. 1 Effect of DDW on the initial growth rate of A4 cells in culture

THE EFFECT OF DDW ON DOGS WITH BREAST ADENOCARCINOMA

The first DDW treated dog (No.1.) was a five-year-old, female, 40 kg bodyweight. The Hungarian breed of shepherd-dog had sufferred from a 10 x 6 cm size exulcerated breast tumour. The tumour had already infiltrated the surrounding tissues and there were five additional small metastases close to the primer tumour. One of these metastases was taken out for histology. Microscopic findings correspond to adenocarcinoma. After consumption of DDW the size of the primer tumour started to decrease (Fig. 2.). When the tumour shrank to 3 x 2 cm the remaining tumour was completely taken out.

D concentration of the blood plasma was measured after nine months' consumption of DDW with 90-95 ppm D content. During that period the 40 kg bodyweight dog had been drinking 130 liter of DDW. The D concentration of the plasma was found to be 122 ± 3 ppm.

The application of DDW on other dogs with breast adenocarcinoma has confirmed our previous results. Fig. 2. shows the changes of primer tumour size of breast tumour in six dogs, including the above mentioned. The results show that there was rapid response in two cases, in three dogs we were able to achieve a 35-67% decrease in tumour size within 2-9 months and in one case the tumour growth had been completely inhibited for 8 months.

The applied dose of DDW with 90-95 ppm D-concentration varied between 0.01-0.02 kg per body kg per day.

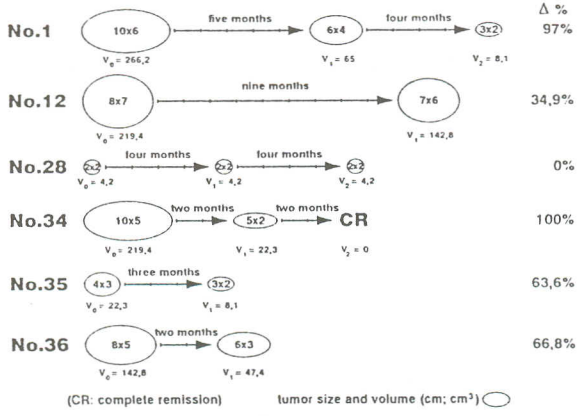

Fig. 2 Effect of DDW on breast carcinoma in dogs

THE EFFECT OF DDW ON HUMAN WITH PROSTATE TUMOUR

Human, phase II double blind clinical trial was initiated two years ago (August, 1995) with the aim of studying the therapeutic effect of ingestion of DDW in patients with prostatic tumour. Until performing the interim analysis (May, 1997) 42 patients were included, seven of the patients were excluded from evaluation, consequently statistical analysis was performed with 35 (19 treated, 16 control).

At the fifth or sixth visit (in the third and fourth month of the clinical trial) the proportion of patients with improving efficacy was statistically significantly greater (5^{th} visit: $p<0.0096$, 6^{th} visit: $p<0.021$) in the treatment group.

The change of prostatic size significantly differs between the two groups: on one hand prostatic volume decreased at the 5% level of significance in the treated group, while score values of the control group can be regarded as unchanged, on the other hand prostatic size decreased in more patients at the 5 % level of significance also in the treated group (Armitage Exact Test: $p<0.015$, Fischer Exact Test: $p<0.011$). This result is confirmed by the observations regarding urination problems, when it was ascertained, that at the 1% significance level more patients had a better judgment of the changes in the symptom in the group drinking DDW (Armitage Exact Test: $p<0.0009$, Fischer Exact Test: $p<0.0018$).

The applied dose of DDW with 90-95 ppm D-concentration varied between 0.014-0.026 kg per body kg per day.

The conclusion which can be drawn from the results of clinical trial is, that the decrease in the deuterium concentration in the patient's organs caused by the DDW may be used as efficacious means in oncologic treatment, but to the proper statistical analysis requires the additional inclusion 50-60 patients into the clinical trial.

DDW STIMULATES THE APOPTOSIS OF A4 INTERLEUKIN DEPENDENT CELL LINE IN VITRO AND OF PC-3 HUMAN PROSTATE TUMOUR CELLS IN MICE

In order to get closer to the mechanism which slows down the tumour growth or leads to tumour regression, we also studied the effect of DDW on mitosis and apoptosis.

The physiological dependence of A4 cells on Interleukin-3 (IL-3) offers an appropriate experimental model system for studying the effect of DDW on cell death since it can readily be induced by the withdrawal of Interleukin-3 from the culture medium. When the cells were exposed to DDW (90 ppm D), Trypan-blue dye-exclusion test of cell viability revealed an increased rate of cell death in A4 cells as compared to their normal counterparts (Fig.3.).

In the experiments with PC-3 tumour cells, following the transplantation of the tumours 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 tumours were removed and histologically examined. 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.

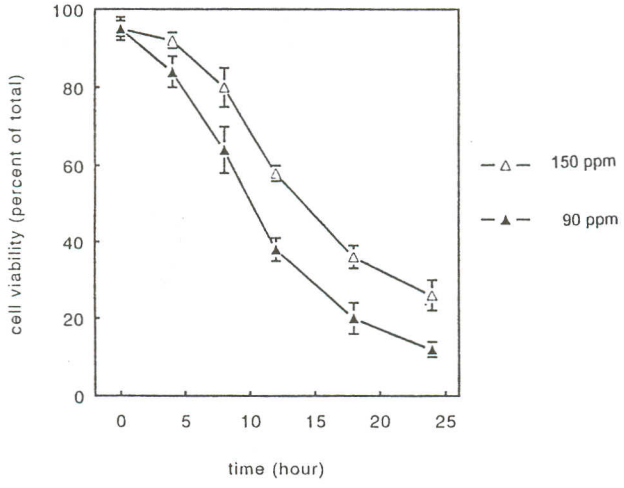

Fig. 3 Effect of DDW on viability of IL-3-deprived A4 cells

CONCLUSIONS

In this communication we present evidence demonstrating that i/ DDW inhibits cell proliferation of A4 and three tumorous cell lines in vitro; ii/ DDW applied as drinking water can induce complete or partial tumour regression in dogs with breast tumour; iii/ interim evaluation of double blind phase II clinical trial indicate the therapeutic effect of DDW on humans; and iv/ DDW can trigger apoptosis in vitro and in vivo. The elucidation of the mechanisms responsible for the inhibition of cell growth or the induction of death of tumorous cells requires further extensive studies.

It is evident from the results that the sensitivity of the different cell lines to the DDW is different. The observation that the effect of DDW on syncronized cells were more

pronounced than on non-syncronized cells may suggest that the importance of D in cell cycle regulation is not the same in the different phases of the cell cycle. It was also found that the lower the D concentration the higher is the inhibitory effect of DDW. We suggest that the effective dosis is 0.014-0.026 kg per body kg per day in human application of DDW with 90-95 ppm D.

The observation that DDW may trigger apoptosis calls the attention to investigate the effect of D depletion on the mechanisms having key role in apoptosis. Similarly we suggest, as it was explained earlier (Somlyai *et al.* 1993), that the cell cycle regulatory system is somehow able to recognize the changes in the D/H ratio and when this ratio reaches a certain threshold this will trigger the molecular mechanism which makes the cell to enter the S phase. We suppose that the decrease of D concentration can intervene in the signal transduction pathways having determinative role in cell proliferation.

We suggest that the application of DDW may open new possibilities in cancer therapy offering a direct intervention into the mechanism playing a central role in cell cycle regulation.

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