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STUDY ON ANTICANCER ACTIVITY OF DEUTERIUM-DEPLETED WATER (DDW) IN EXPERIMENTAL ONCOLOGY

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Abstract

The authors demonstrated the effect of deuterium depleted water (DDW) with a concentration of 60 ppm to be a very good inhibitor of the neoplastic cell proliferation in outbred Wistar rats inoculated with two strains of the highest tumor aggressiveness (Walker 256 and T8 Guérin).
For this experiment were organized groups, in equal parts by sex of outbread Wistar rats, with an average weight of 100g, which were established both control groups and experimental groups.
Through the experiment, animals were monitored in anatomoclinical terms. Laboratory tests were executed periodically, especially cytomorphological. Dead animals were subjected to a careful pathological examination.
It is well known that high malignant Walker 256 and T_8 Guérin tumour strains develop a solid, ulcerated subcutaneous cancer, after an incubation of 5-6 days. The reproductibility of this type of cancer is of 95%. Therefore the death of the rats occurs within a short period of 40-60 days. The cumulative effect of DDW 60 ppm on the rats grafted with Walker 256 and T_8 Guérin strains was about 28-30 %. This percentage comprises both the animals in which the effect of primary reject of the tumour graft was noticed and the healing effect after an important development of the tumour.

Key words: cancer, DDW 60 ppm, experimental, oncotherapy.

INTRODUCTION

Regarding the application of deuterium-depleted water (DDW) effects in biology, oncology and therapy, the first theoretical and laboratory data are found in the papers provided by Somlyai G. et al, Bild W. et al and Gyöngyi Z (1998-2002) followed by well-known research data in other scientific papers. (1, 2, 3, 4, 5, 6). Many attempts to cure Wistar outbred rats, inoculated with high malignity 256 Walker and T_8 Guérin strain tumour cells, by means of various therapies, within the Cancer Biology Department from the Oncological Institute Bucharest (IOB), were unsuccessful; therefore, this experiment was carried out in order to provide a new therapy.

Therefore, as all these therapies were ineffective, DDW was taken into account, with different deuterium concentrations, in relation to the biological components of normal and/or pathological life.
Consequently, DDW with a deuterium concentration of 60 ppm (DDW 60 ppm) was chosen in order to carry out the experiment, due to the previous results showing its possible anti-cancer protection, superior to all other deuterium concentrations.

MATERIALS AND METHOD

500 Wistar outbred homogenous rats from IOB own lot, all with an average weight of 100 g, males and females in equal parts, were used. These were divided in two groups, initially as follows: control group (250 rats), which drank exclusively tap water (TW) with a deuterium concentration of 150 ppm (TW 150 ppm); and experimental group (250 rats), drinking exclusively deuterium-depleted water (DDW) with a deuterium concentration of 60 ppm (DDW 60 ppm). Differences between the initial number of 500 rats in the experiment and the experiment onset values of 320 rats are due to the following facts:

1. some rats had to be taken from the control group, in order to carry out other experiments, therefore only 100 rats were left for this experiment;

2. from the initial experimental group consisting of 250 rats, only 220 out of 250 rats remained alive at the experiment onset – the rest representing mortality due to an enterotoxaemia.

DDW used in the experiment was provided by the National Research and Development Institute for Cryogenics and Isotopic Technologies - ICSI Rm. Valcea.

The daily water intake was approximately 15 ml/day. After 60 days, considered "preparatory" for the experiment, the animals were divided in four study groups, as follows:
Groups one and two were subjected to latero-dorsal subcutaneous grafting with 1 X 10^7 ascites cells in 0,5 ml of normal saline solution of 256 Walker (ascitogenous) (tumour cells with a viability over 98%). Viability determination for the tumour cells to be grafted was evaluated by counting the dead cells (trypan blue stained) under microscope, and calculating the ratio corresponding to 1000 cells displayed on the slide.

Group one (experimental) – consisting of 150 rats which drank DDW 60 ppm, both prior to (over 60 days), and during the entire experiment.

Group two (control) – consisting of 50 rats which drank exclusively TW 150 ppm, during the entire experiment.

Groups three and four were subjected to latero-dorsal subcutaneous grafting, with a saline suspension of ascites tumour cells from T_8 Guérin strain with 1 X 10^7 tumour cells with 98% viability percentage. Viability determination for the tumour cells to be grafted was evaluated by the same method (trypan blue stained).

Group three (experimental) – consisting of 79 rats which drank DDW 60 ppm exclusively.

Group four (control) – consisting of 50 rats which drank exclusively TW 150 ppm.

The food ingested by the rats was consistent with IOB bio-basis standards, during the entire experiment.

The surveillance protocol of the four groups included:

Graft status was assessed daily by inspection and palpation in all animals, beginning with day four post-grafting, in order to identify and note the onset day of malignant neoformation; furthermore, this day represented the subcutaneous grafting day. Consequently, after identification of the tumour, the tumour growth was evaluated objectively by measuring the two perpendicular axes (major and minor). Also, the tumour volume was calculated daily using the formula:

$$V = 0,4(constant)\ X\ (a\ X\ b^2),$$

where – V represents the volume in mm^3 ; a, and b represent the values of the two axes, namely major (a) and minor (b).

The animals were weighed daily, monitoring their weight evolution, and clinical assessments on the grafted animals were carried out in order to note any change in their clinical status. Mortality was recorded daily, as well.

Cytomorphological examinations were performed periodically in order to establish the leukocyte formula, and to identify the "*blast cells"*, as well as dendritic and NK cells, followed by cytological evaluations from haematopoietic bone marrow and lymph nodes.
The following assessments of the tumour growth were performed periodically, usually on a twenty-days basis, according to NCI criteria (National Cancer Institute – Bethesda, USA - 1990), by calculating:

- tumour growth index (TGI %), provided that values of 50% and above represented a significant inhibition of tumour growth;

- mean survival time (MST) of the grafted animals;
- T/C ratio (%) (T = treated, C=control X 100); the values had to be > 125;
- it was very important to assess the "*survival time* (ST) *increasing*", which had to be over 25%, evaluated in days, since the tumour graft onset, and until the death of the last animal from each group or until the maximum day of the experiment (700 days in our case);
- establishing the number of long term surviving animals, versus number of treated animals.

Note that standard operating procedures (SOP) were fully respected in order to validate the good manufacturing practices (GMP) currently used in pharmacological studies. Consequently, for quantitative estimation of the anti-cancer effect of the product, two variables required by GMP were taken into account:

- - the independent prediction variable, therefore minimum 1 X 10^6 tumour cells must be present in each tumour grafting, under the same standard terms;
- - the dependent prediction variable – concerning the following criteria:
- -latent period (in days) – representing the number of days from grafting until the smallest tumour growth onset;
- -tumour incidence, estimating the number of animals with/without tumours, tumour rejections;
- -mean survival time (MST), representing the days since grafting, and until the death of the last animal in the each experimental group.
- -monitoring the physiological status of the animals:
- -weekly weighing of the rats;
- -monitoring of water and food consumption of the animals in every group;
- -performing complete haematological exams; these consisted of Coulter Counter cell counting, whole blood and leukocyte concentrated (LCT) blood smears, in order to establish the leukocyte formula;
- -post-mortem examinations of the animals (necropsy), including lymphadenograms and medulograms, in order to notice the local development of the tumour, with or without metastasis of lymph nodes; ratio of virgin lymphocytes (antigenic unexposed) and those antigenic exposed; furthermore, the ratio between total immunologic competent cells and mast cells was evaluated.

RESULTS AND DISCUSSIONS

The results of this study are relevant as all the rats in the "*control groups"* - which consumed TW 150 ppm - grafted with both T_8 Guérin, and Walker 256 strains tumour cells, died within a post-grafting period of 40-60 days. In conclusion, their death was due to some malignant ulcerous and invasive tumour development, including regional lymph nodes metastasis. On the contrary, the groups grafted with the same tumour cells, but which consumed exclusively DDW 60 ppm for "*a long time*", recorded exquisite results as shown in tables 1 and 2.
Therefore, our experiment managed to emphasize some specific actions of DDW 60 ppm in comparison to TW 150 ppm, as follows:

1. The onset of inoculated graft and its anatomoclinical visualization increased from 5-6 days in the control group to 10-12 days in the groups of animals that consumed DDW 60 ppm for 60 days pre-grafting.

2. A DDW 60 ppm consumption exclusively for 700 days was shown to have a beneficial effect on the animals, consequently leading to an *"immunologic boom".*

3. Furthermore, a broad antineoplastic activity, both by initiation and completion of "*graft early reject*", and an increase in neoplastic development time, for both tumour strains, was demonstrated by the experiment. Thus, both grafted tumour strains were lysed

either early or late leading to a less intended survival time of approximately 28% from the total grafted rats, over the entire experiment period of 700 days. In conclusion this result is compared with the 100% morbidity of the control groups within a short post-grafting period of 40-60 days.

4. The experiment demonstrated that these actions are based on an intense activity of immune modulation of "*the cellular immune system*" (CIS) by clonal proliferation of lymphocytes B, of NK cells, and of the dendritic-mast system. This activity was identified both in haematopoietic bone marrow, lymph nodes, and peripheral blood.
Our results are broadly similar to data from specialized literature.

Our experiments brought some novelties concerning:

- Demonstration of no toxic effect or life discomfort for the animals in the experimental groups, under the exclusive administration of DDW 60 ppm, for a long time, to unusual digits such as 700 days.
- Exclusive administration on the long term of DDW 60 ppm allowed the identification of primary graft reject of the tumour graft in about 20% of inoculated rats, on the one hand, and on the other hand, achieved a significant therapeutic effect in about 10% of inoculated animals providing that the inoculated and developed tumour was lysed in time, thus leading to a very good healing of the tumour site.
- The extension of the time in which the smallest post-grafting tumour evolved, from 5-6 days for control group, to 10-12 days for experimental groups (thus proving the graft attachment)
- In the control group, after 5-6 days following the tumour onset, there could be noticed the tumour development and subsequent death of all the inoculated rats occurred within 40-60 days.

Table 1. Summary of the experiments general data

Inoculated rat line	No. of days with DDW 60 ppm before grafting	No. of post-grafting days after the tumour growth onset	No. of animals			Remarks
			total	males	females	
Walker 256	60	11	150	75	75	Experimental group
Walker 256	-	6	50	45	45	Control group
T8 Guerin	60	11	79	40	39	Experimental group
T8 Guerin	-	6	50	25	25	Control group

Table 2. Summary of the final results regarding the experimental anticancer value of DDW 60 ppm in Wistar outbred rats

Grafted tumour cell line	No. of animals experimental group/ control group	Dead animals control group during 40-60 days	T_8 Guerin grafted surviving animals over 500 days	Walker 256 grafted surviving animals over 500 days	Remarks
Walker 256	150/50	50	-	28,5%	Morphoclinical assessments were conducted on an 100 days basis over the 700 experimental days
T_8 Guerin	79/50	50	28%	-	

CONCLUSIONS

The most important effect of DDW 60 ppm consumption for 60 days before tumour grafting was to induce the graft primary reject in about 20% of the inoculated animals.
Consequently, the intake of DDW 60 ppm for 60 days in advance of the experiment, and afterwards for 700 days, showed an inhibiting

action of the cancer development in the subcutaneous inoculated Wistar outbred rats with Walker 256 and T_8 Guérin strains at the rate of approximately 8 – 10 %.
The cumulative effect of DDW 60 ppm on the rats grafted with Walker 256 and T_8 Guérin strains was about 28-30 %. This percentage comprises both the animals in which the effect of primary reject of the tumour graft was noticed and the healing effect after an important development of the tumour.
DDW 60 ppm showed a special activity of "*immune modulation"* of the cellular immune system (CIS), by clonal stimulation of mast-dendritic system, of NK cells, and lymphocytes B;
Therefore, note that DDW 60 ppm produced no toxic or inhibiting effect to the animals even in the long run consumption (700 days) exclusively.

Fig.1 rat with tumor in flank area after a tumoral inoculation

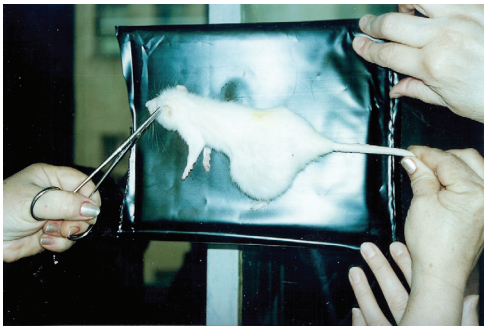


Fig.2 tumor ulcera after 40 days

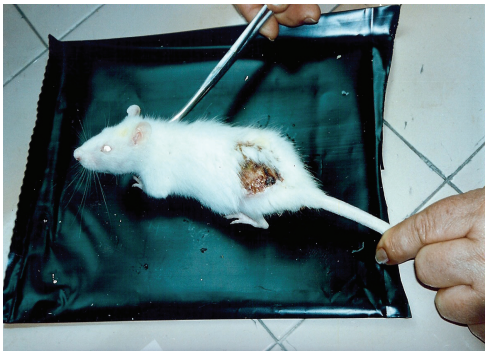
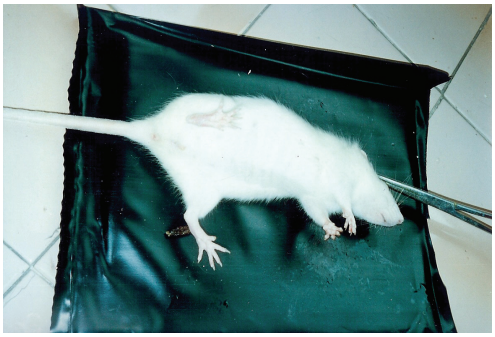


Fig.3 day 70-reepithelization of the ulceral area



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