Bulletin USAMV-CN, 64/2007 (1-2).

THE STUDY OF SOME CLINICAL AND HAEMATOLOGICAL PARAMETERS ON THE WISTAR RATS INOCULATED WITH WALKER 256 ASCITE CORRELATED TO THE DAILY DDW CONSUMPTION*

Marcus I*, D. Mărculescu[**], B. Sevastre[*], Daniela Zinveliu[*], Gh. Pop[***], Al. Pop[*]

*USAMV Cluj Napoca. Faculty of Veterinary Medicine Manastrur street, nr. 3-5. E-mail: ioanbmarcus@gmail.com
**RAAN Drobeta Turnu-Severin. E-mail: office@raan.ro
*** DSV Oradea. E-mail: ghpop66@gmail.com

Key words: Walker 256 ascite, deuterium depleted water, Wistar rats, blood investigations

Abstract. The aim of this experimental study was to evaluate, from the clinical and laboratory point of view, the effects of the daily deuterium depleted water intake on the Wistar rats inoculated intraperitoneum (ip) with Walker 256 ascitic carcinoma. The experiment was performed on 20 Wistar rats, male adults, divided in four groups, each of them containing 5 animals. In accordance to the experimental protocol, the animals from groups 2 and 4 were administrated *ad libitum* with deuterium depleted water for a period of six months, and then were inoculated (group 4) intraperitoneum (ip) with Walker 256 ascitic carcinoma (between 85 000 – 90 000 tumour cells/ml ascite liquid). During the first stage of the experiment (1 to 6 months) all experimental animals were weekly measured for the evolution of the body weight and some other clinical aspects, regarding the food and water intake, respectively some peculiarities of the behaviour and the general health status. After intraperitoneum inoculation of the Walker 256 ascitic carcinoma all animals were rigurously observed and were weekly evaluated from the the body weight evolution, respectively of some other abnormal manifestations point of view. Finally, the animals were killed after 5 weeks from the intraperitoneum inoculation with Walker 256 ascite carcinoma under narcose with ether, by cardiac punction, and blood samples were collected. The values of the main heamatological parameters from the blood samples (RBCs, Hb and Ht) were evaluated, and also subsequent from the plasma obtained by centrifugation were measured the values of some biochemical compounds, as plasma proteins, albumins, globulins, cholesterol, glucose, urea and creatinine. The obtained results permit us to come to the conclusion that daily deuterim depleted water consumption influences, more or less significantly, the level of the clinical and haematological parameters investigated in this experiment, both in the healty Wistar rats and the animals inoculated intraperitoneum with Walker 256 ascitic carcinoma.

INTRODUCTION

Recent studies in the biomedical domain have proved that there is a strong dependence between the deuterium content of water and the live cells' growth vellocity (*Altermatt, 1988*). Using deuterium depleted water instead of that with a natural content o deuterium it was observed through experiments on laboratory animals a significant decrease in the growth rate of the malignant cells and the inhibation of transplant tumor development *(Samlay et all, 2001).* In conformity to the same studies, only a smaller content than *40 ppm* in deuterium of superlight water favours the therapeutic action. The favourable results obtained in this domain in different centers of cancer research determined that in the year 2002 for Romania to introduce deuterium depleted water in the clinical use, as a way of experimental/spontaneous tumor development control in animals/humans.

[*] *This study was supported by a CNCSIS Grant, type A, Thema 11/2005, Cod CNCSIS 790 and with the kindly assistance of RAAN, Drobeta Turnu Severin.*

Deuterium is the heavy isotope of hydrogen, which was discovered in 1932 by Harold Crayton Urey. The deuterium depleted water can be produced by boiling the water many times or rather with electrical fission *(Katz and Crespi, 1970, Somlyai et all, 1993).* In this process a lot of hydrogen gas springs up, which must be burned. In absence of deuterium, cells get some kind of shock, but while the healthy ones can adapt very fast to the new situation, the tumorous ones can't, so they die *(Zimermann et all, 1973).* Researches with mice in which human tumor was transplanted proved the same thing *(Somlyai et all, 1993, Ieremia et all, 1997).* In several cases it was sustained and proved the fact that deuterium depleted water inhibits the tumor cells' development in fibroblastic cultures. In the experiments performed on laboratory animals, the elimination of deuterium from the organism was made in steps, by offering the water to the mice. Surprisingly, replacing normal water with the deuterium depleted one led to the inhibition of malignant tumor development *(Lamprect, 1990; Altermatt, 1990).* At the beggining of the '90s it was descovered that decreasing the concentration of deuterium in the organism, under the physiologic level, slows the progression of different tumor cells in mice and the survival rate extends *(Somlyai et all., 2000; FEBS Letter, 1993).* Decreasing the concentration of deuterium in the organism can be accomplished by consuming deuterium depleted water (DDW) and different others deuterium poor nutrients. Thus, all these led to the conclusion that the DDW can have therapeutic properties in pacients with cancer or suffering from other neoplasic disorders. In the experiments performed in the laboratory immunosupressed mice were used, grafted with malignant cells from humans, and in which the following aspects were noticed after consuming deuterium depleted water: the rate of cell proliferation was reduced by 30% in the first 20 hours in cell cultures obtained from animal tissue, in vitro; at 80 days the inhibited cell proliferation was observed in mice for 70% and for 59% from the cases in which the tumours had really evolved, their regression was observed in time, even their dissapearance (*Somlyai et all, 2000).* In many other experimental studies made on transplantable tumors in mice and rats the size of tumors stagnated or decreased as a result of deuterium depleted water intake (Marcus et all., 1996, 1997).

MATERIAL AND METHOD

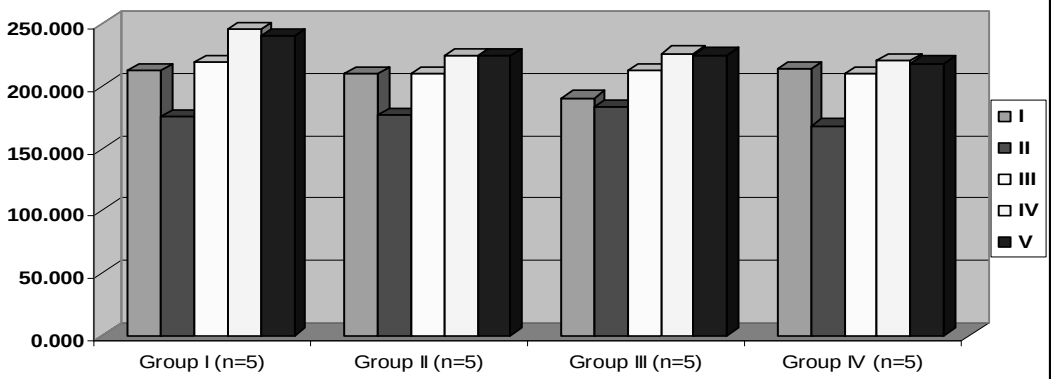
The experiment was performed on 29 adults Wistar rats, divided in four groups, each of them containing 5 animals. Group 1 (n = 5), was used to obtain the reference values for the clinical, haematological and biochemical parameters investigated, and had at the beggining of the experiment a body mss of 212,4 ± 13,06 g/animal. Group 2 (n = 5) with an initial body mass of 210 ± 14,89 g/animal, was used for the investigation of the biological effects of deuterium depleted water intake on healthy animals. Group 3 (n = 5) which had a body mass at the beggining of the experiment of 190,4 ± 11,82 g/animal, was inoculated intraperitoneum (ip) with 2 ml of Walker 256 ascitic carcinoma. Group 4 (n = 5) with a body mass at the beggining of the experiment of 213,8 ±29,8 g/animal, was inoculated ip. with 2 ml of Walker 256 ascitic carcinoma and subsequent was drinking the deuterim depleted water for the entire period of the experiment. During the whole period of experiment, all the animals were evaluated from the clinical point of view, by weekly measurement of the body mass, tumour volume and some aspects of their behaviour. After 7 months from the beggining of the experiments, the animals were sacrificed under narcose with ether, by cardiac punction, and the blood samples were gathered. From the samples obtained from animals the values of the the main heamatological parameters from the blood samples (RBCs, Hb and Ht) were measured, also subsequently from the plasma samples the values of some biochemical compounds, as plasma proteins, albumins, globulins, A/G ratio, cholesterol, glucose, urea and creatinine were investigated. All the

results obtained were statisticaly processed, by determination of the arithmetical mean, standard deviation, the percentage differences (%) and the „t" student test.

RESULTS AND DISCUSSIONS

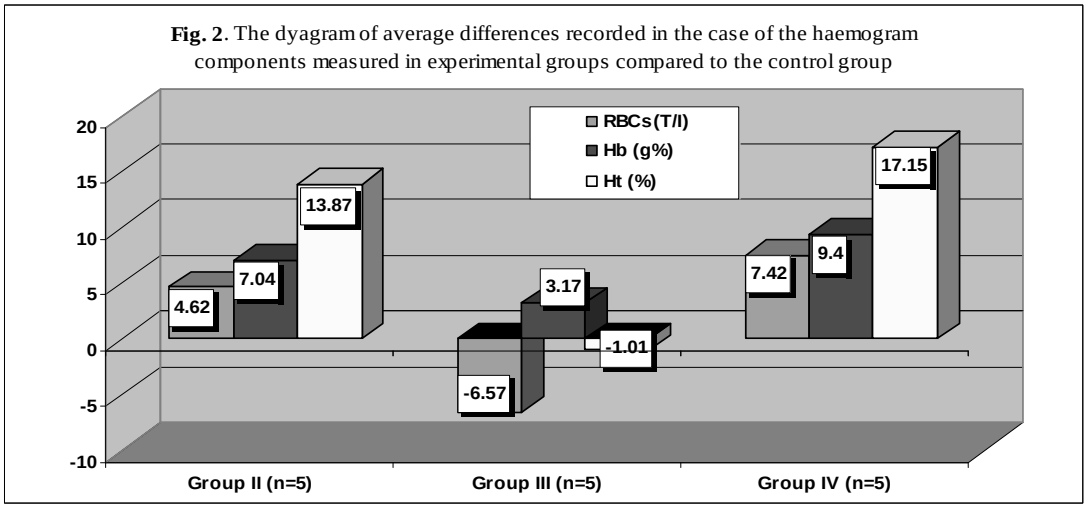
From the examination of the results presented in the figure 1, one can observe that the body weight in the case of the reference group (group 1) and of the group inoculated with Walker 256 ascitic carcinoma (group 3), increase gradually during the experimental period. One the other hand, in the case of the groups 2 (DDW) and 4 (DDW + W 256), which were drinking deuterium depleted water, the body mass didn't record very expressive differences at the end of the experimental period, comparative with the value recorded at the biggining of the experiment.

Fig. 1. The dyagram of the body weight evolution in the experimental animal groups on the entire experimental period (months 1-7)



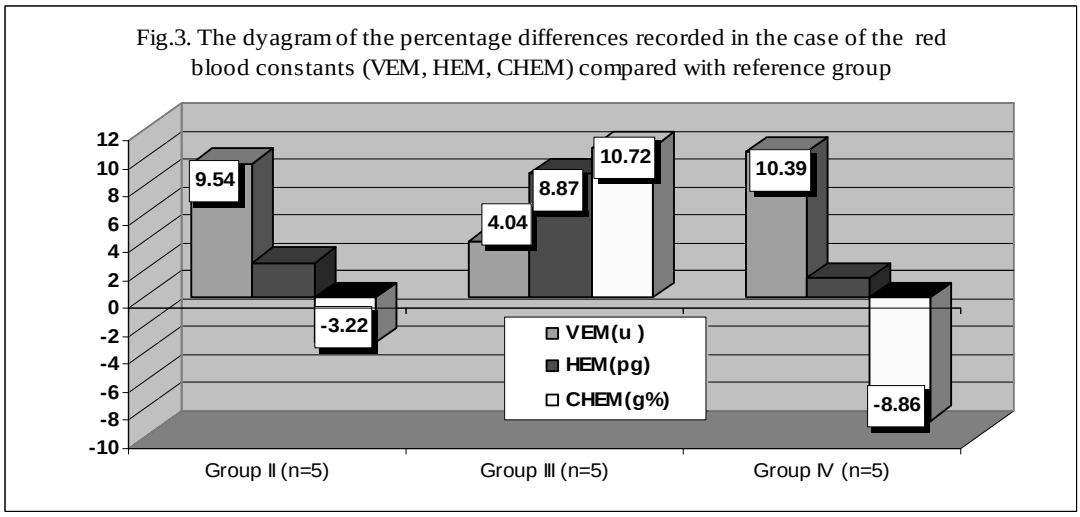
In the case of the haemogram components investigated (fig. 2), the results obtained in the reference group (1) were situated between the normal fluctuation for this kind of animals.

Fig. 2. The dyagram of average differences recorded in the case of the haemogram components measured in experimental groups compared to the control group

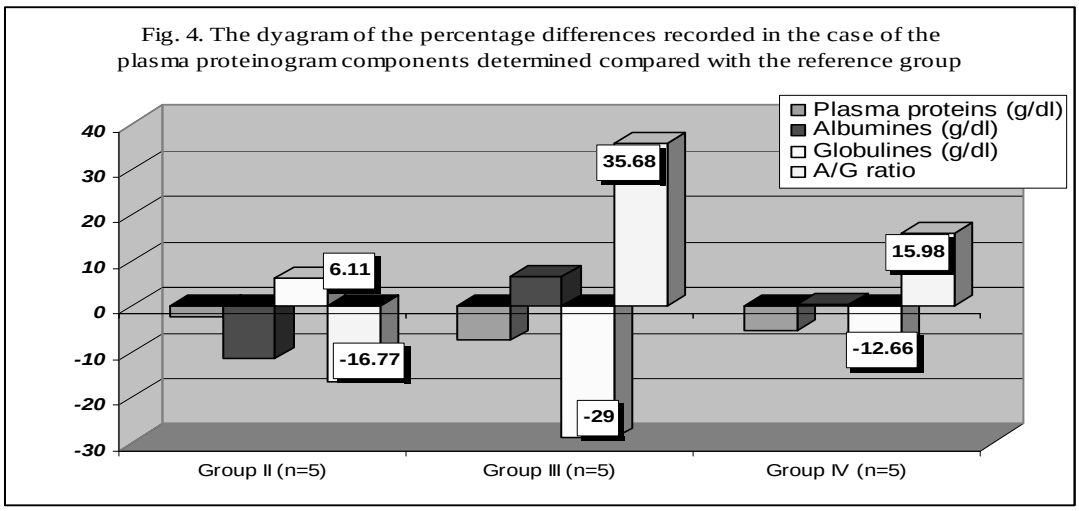


By comparison with the results obtained in the reference group 1, the percentage differences recorded in the case of determinations made in the animals inoculated with Walker 256 ascitic carcinoma, respectively in those which consumed deuterium depleted water, the following aspects can observed: the possitive percentage differences for all the components of the haemogram (RBCs +4,62%, Hb +7,04%, Ht +13,87%) in the group 2, aspect which can be observed in the case of the group 4, too (RBCs +7,42%, Hb +9,4%, Ht

+17,15%). The way and the intensity of the variations that affect the components of the haemogram were not the same for the blood red cells constants, VEM, HEM, CHEM, (fig.3).



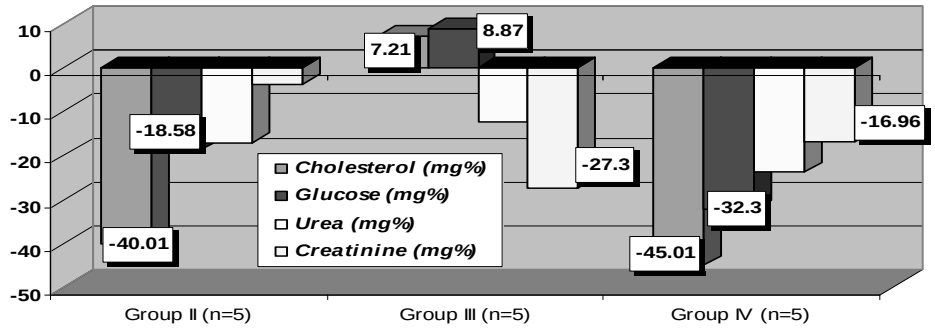
In the next figure (fig. 4) the diagram of the percentage differences that affect the level of the plasma proteinogram components is presented, respectively of the plasma proteins, albumins, globulins and albumins/globulis ratio. One can observed that there aren't expressive variations of the plasma proteins concentration in the experimental groups 2, 3 and 4 comparatively with the reference group 1. But, in the case of the plasma globulins level the percentage differences recorded are negative for the groups 3 (-29%) and 4 (-12,66%), fact that will determine larger fluctuation of the A/G ratio (+35,6% in group 3 and +15,9% in group 4).



As following (fig. 5) the concentration of some plasma biochemical components were determined, as cholesterol, glucose, urea and creatinine. As well as in the case of the plasma proteins, the results obtained by determining of these plasma biochemical compounds in the reference group (1) are situated in the normal limit of fluctuation for this species (Ghergariu et all, 2000, Marcus, 2004). Compared to the reference group 1, all the biochemical components investigated recorded negative fluctuations for all experimental groups, but the most expressive differences affected the values of the cholesterol and glucose, especially in the case of the groups 2 and 4, which consumed deuterium depleted water.

These percentage differences are strongly negative both for cholesterol (-40,01% in group 2 and – 45,01% in group 4) and glucose (-18,58% in group 2 and – 32,3% in group 4).

Fig. 5. The dyagram of the percentage differences recorded in the case of plasma biochemical parameters for experimental groups compared with reference group



One indirect proof of the presence of certain tumors is the appearance of specific proteins (tumor markers) in the blood. After the consumption of deuterium depleted water the value of this kind of tumor markers decrease *(Blaga et all, 1978).* This decrease is connected to the decrease of tumor volume, which also reinforces the anti-cancer effect of deuterium depletion. Several examples from the literature have shown that in total remission in some cases there was no relapse for years when the patient had been drinking deuterium depleted water, but when the consumption was stopped, the tumor appeared again within several months. This reveals that deuterium depleted water helped these patients maintain an internal balance and inhibited tumor growth *(Blaga et all, 1978, Somlyai et all, 1993, Ieremia et all, 1997).*

CONCLUSIONS

- Long term administration of the deuterium depleted water doesn't develop any adverse effects in animals, excepting the fact that it interfere with the rate of body development.
- The deuterium depleted water is also able to downregulate the proliferation of the malignant cells. This effect consists in decreasing the rate of the tumoral growth, aspect expressed either by a small tumor volume or by the absence of the tumor development in the animals which have consumed DDW, compared to the tumor control group.
- From the haematological point of view, in the case of the all investigated parameters (RBCs, Hb and Ht) increased values can be observed in the case of animals which have consumed deuterium depleted water, compared to the reference group. This result contradicts the curent literature data, in conformity with which deuterium depleted water would action through mitosis inhibation at the level of high multiplication rithm cell populatations.
- The constantly low values of the biochemical components investigated (cholesterol, glucose, urea and creatinine) in the animal groups which have consumed deuterium depleted water, suggest a cell metabolism inhibation effect, aspect expressed by significantly negative values compared to the reference group.

BIBLIOGRAPHY

1) ALTERMATT H.J., 1990, Deuterium Isotope Effects in Chemistry and Biology'', International Journal of cancer, 475- 480.

2) BERDEA P, CRISTINA DOBROTA, C. COSMA, STELA CUNA, 1999, Growing rate decrease of the maize embryos sprouted in deuterium-deplated water (20 ppm), Colloqvium Spectroscopicum Internationale XXXI, Ankara, Turkey, Sept. 5-10,

3) BERDEA P, STELA CUNA, M.CAZACU, M.TUDOSE, 2001, *Deuterium variations in human blood serum,* Studia UBB, PHYSICA, SPECIAL ISSUE, 256 – 258.

4) BLAGA L, LUCIA BLAGA, 1978, The deuterium content of human metabolic fluids in relation with human metabolic processes (in Romania), IFA-Bucuresti, Scientific Report, 1-4

5) DAVID SMITH, 2001, Rapid measurement of deuterium content of breath following oral ingestion to determine body water'', Physiological Measurement, Vol.22,

6) KUSHNER DJ, ALISON BAKER, T.G.DUNSTALL, 2001, Biotechnological potential of heavy water and deuterated compounds'', University of Toronto, Canada.

7) GHERGARIU S, MARINA SPANU, AL. POP, L. KADAR, 2000, **Manual de Laborator Clinic Veterinar**, Ed. All Educational, Bucuresti, 34-67.

8) GOODALL KIRK.B, 2003, Preliminary Analysis of Deuterium's Role in DNA Degradations'(www.hydros.com/prod-vet.phtml).

9) IEREMIA D, RODICA DUMITRESCU, ELENA NES, 199, Restriction of cellular growth by deuterium deprivation, IV-th Nat.Conf. of Biophys., 16-18 Oct. Cluj-Napoca

10) LAMPRECT J.,1990, Mitosis Arrested By Deuterium Oxide light microscopic, immunofluorescence and ultrastructural characterization'', Europian Journal of Cell Biology, Stuttgard, Germany, April; 303-312.

11) MARCUS I.B.,2004, Biologia şi patologia animalelor de laborator'', Ed.Risoprint, Cluj-Napoca; 69-82.

12) MARCUS I, B. SEVASTRE, A.N OROS, MIHAELA DUMA, AL. POP, 2005, Cercetări privind efectul consumului de apă sărăcită în Deuteriu (Deuterium Depleted Water) asupra unor particularităţi clinice şi hematologice la şoareci NMRI grefaţi ip. cu ascita experimentală Ehrlich, *«Al VI-lea Congres Naţional de Farmacologie, Terapeutică şi Toxicologie Clinică, cu participare internaţională»*, UMF Cluj, 130, ISBN 973-693-121-8.

13) MARCUS I, L FARCAL, AL POP, B SEVASTRE, A OROS, 2005, The Deuterium Depleted Water intake correlated with the values of some haematological, biochemical and gravimetric parameters in NMRI mice inoculated with a transplantable tumor (Ehrlich ascite), Bul. USAMV-CN, Vol. 62-2005, 172-176, ISSN 1454-2382.

14) MARCUS I, B. SEVASTRE, AL. POP, Daniela ZINVELIU, P. BERDEA, 2006, Investigation concerning the values of some biochemical plasma compounds in Wistar rats grafted iv. with a transplantable tumour (Walker 256 ascitic carcinoma), correlated with the daily dauterium depleted water intake, Bul. USAMV CN, Vol. 63, 441-442, ISSN 1454-2390.

15) KATZ J.J AND H.L. CRESPI, 1970, Isotope effects in biological systems in: C.J. Collins and N.S. Bowman (eds.), Isotope effects in Chemical Reactions, Van Nostrand Reinhold Company, New York,. 286-353.

16) SOMLYAI G, G. JANCSO, G. JAKLI, K. VASS, B. BARNA, V. LAKICS AND T. GAAL, 1993, Naturally occurring deuterium is essential for the normal growth rate of cells, FEBS 317, 1-4.

17) SOMLYAI G., 1998, The biological effect of deuterium depleted water, a posibile new tool in cancer therapy', Z. Onkol./J. of Oncol, 23-28.

18) ZIMERMANN U, AND U. CEGLA, 1973, Der Deuterium-und Sauerstoff-18-Gehalt der Korperflussigkeit des Menschen und seine Anderung bei Ortswechsel, Natur wissenschaften, **60**, , 243-246.