Heavy Water (D_2O) Inhibits the Growth of C6 Gliomas

M. Benito[1], P. Sanchez[1], T. B. Rodrigues[1], A. Sierra[1], S. Garrido[1], P. López-Larrubia[2], S. Cerdán[3]

[1]NMR Lab, Instituto de Investigaciones Biomedicas "Alberto Sols", Madrid, Spain, [2]SIERMAC, Instituto de Investigaciones Biomedicas "Alberto Sols", Madrid, Spain, [3]NMR Lab, de Investigaciones Biomedicas "Alberto Sols", Madrid, Spain

Introduction

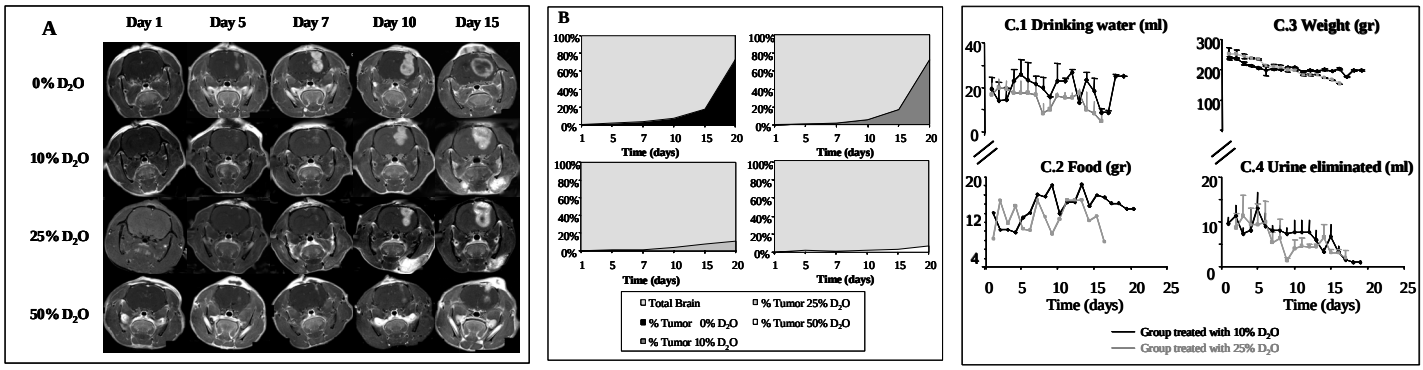
Experiments with D_2O started in the early thirties[1], describing the effects of deuterated water on human metabolism[2]. D_2O was soon reported to affect cellular growth and differentiation and to elicit important antimitotic effects[3, 4]. Interestingly, Barbour and Allen reported in 1983 that deuterated water retarded tumor growth in mice, but further experimentation in this topic ceased because the development of collateral toxic effects[5, 6]. With the aim to reevaluate dose-response profiles and toxicity this report examines the effects of D_2O on the growth of C6 gliomas using conventional MRI.

Materials and Methods

Four groups (n=4) of female Wistar rats were used in these experiments. Their weight was between 235-250 g. and was determined daily during experiment (Figure C.3). Prior to C6 cell implantation, the animals were anesthetized intraperitoneally with a mixture of Ketolar, Valium and Atropine (1ml/gr.rat). Then, 10^5 cells were implanted stereotaxically in their right brain at 3.5 mm from Bregma and 5.5 mm of depth from the cranium. One group was kept as control while the others were treated with different concentrations of D_2O (10, 25 and 50% v/v) in the drinking water. Rats were placed in metabolic cages to determine precisely the amounts of water drunk, food ingested and urine eliminated (Figure C.1, C.2 and C.4). MRI measurements were performed on a 7 T horizontal-bore magnet (16 cm bore) interfaced with a Bruker Pharmascan console, using a 7 cm commercial Alderman-Grant resonator. Anesthesia was initiated in an induction box with a mixture of isofluorane/oxygen (2%, 2.0 ml/h) and maintained (1.2 ml/h) throughout the MRI examination. T_1 and T_2 weighted images (9 slices 2mm, TR: 500/2500 ms, TE: 15/60 ms, respectively) were acquired (pre and post-contrast) in all animals to follow the growth of the tumor during fifteen days. To administer contrast, the tail vein was catheterized (Abocad 24G) and GdDTPA (Magnevist, Schering) was injected to reach a dose of 0.2 mmol/Kg. The relative tumor volume was determined as compared to the total volume of the brain, accounting for the interslice distance (2.5mm).

Results

Figure A depicts representative T_1 weighted MRI images from the different animal groups as treated with increasing concentrations of D_2O (v/v) in their drinking water. **Figure B** represents the changes in relative tumor volume for the different concentrations of D_2O in drinking water. It is apparent that D_2O delays or even aborts tumor growth. D_2O decreased slightly water intake, urine elimination and body weight, without significant effects on food intake (**Figure C**). During this period animals maintained normal hair color and did not show symptoms of hyperexcitability or locomotor defects.



Conclusions

During the first days after C6 cell implantation, tumor growth appears to be linear, becoming exponential ten days after. In particular, the tumor volume increases exponentially from 7% (day 15) to 92% (day 20). Treatment with D_2O in drinking water, slowed down tumor growth in a dose dependent manner (**Figures A and B**) without apparent signs of toxicity, during these short periods. The mechanism of this effect remains to be explored, although it could involve limitations in the proton linked transporters since deuterons have been reported not to substitute for protons in some of these processes[7].

References

1. Lewis, G.N. (1934) Science (Wash D.C.) 79:151-3
2. Heveys, G. Hofer, E. (1934) Klin.Wchschr.13:1524
3. Gross, P.R., Spindel, W. (1960) Science 159:37-39
4. Gross, P.R., Harding, C.V. (1961) Science 133:1131-1133
5. Barbour, H.G., Allen, G.(1934) Amer. J. Cancer 32:440-446
6. Katz, J.J. Crespi, H.L., Hasterlik, R.J. Thomson, J.F., Finkel, A.J. (1957) J. Natl. Cancer Inst. 18:641-659
7. Kotyk, A., Dvorakova, M., Koryta, J. (1990) FEBS Lett. 264-2: 203-205