



2004 Examination of the effects of high oxygen content water on tumor cells

During our experiments we examined the encumbering effects on tumour cells of oxygenated water, that is KQN water, with mice tumour lines.

Growing of cell lines

We used 2 cell lines (H59, LLT- HH) in our experiments. The H59 is less malignant, while the LLT-HH is a Lewis Lung tumour line with a strong metastatic ability. There is a separate documentation on its deposition. We grew the tumour cells in RPMI-1640 nutritive solution, we added 5-10% of FCS (GIBCO) 0.01 M HEPES and 2×10^{-3} M of glutamine and we grew the tumour cells for 4-10 days in it for the purpose of the experiments.

MTT assay

We determined the number of the tumour cells with the aid of the following material: MTT (3-(4,5-dimethyl thiazol -2 -yl -2-5-diphenyltetrazolium bromide) assay. The measurement is based on the phenomenon that in living cells the tetrazolium ring breaks off from the light yellow MTT and as a result dark blue formazan crystals form, which are not permeable for the cell membrane, so they accumulate within the living cells. . Consequently the number of the living cells is directly proportional to the amount of the formazan derivant. The colour blue can be detected after the solubilization of the cell membrane with colorimetry. The result that is the extinction can be read by the ELISA reader at the wave length of 550 nm. The measured extinction is directly proportional to the number of the cells. We demonstrate the extinction in our experimental results.

The order of the experiments was the following:

- A. The counting of cells, determination of their viability and the distribution of them onto pates.
- B. On the indicated days the treatment or change of the nutritive solution
- C. Sampling between the 5th and 7th days after the transplantation
- D. Measurement of the extinction of the treated and untreated groups with the aid of MTT assay. The decrease of the extinction value shows the encumbering effect of the high oxygen content water (OGV) on tumour cells.

: KQN = KAQUN WATER

The experiments performed

Experiment 1.

In the first experiment we transplanted 10^3 tumour cells to a plate with 24 holes. We mixed the nutritive solution with the oxygenated water in different concentrate. We treated the control group with the appropriate amount of distilled water. We determined every time the oxygen content of the water and recorded it in a table.

We assessed the results of the experiment on the 6th and 7th days with the aid of the MTT assay.

Experiment 1 The effect of OGV on the tumour cells

(OGV = high oxygen content water, DV = distilled water) 3 treatments on the 1st, 3rd and 4th days, assessment on the 6th day

Groups No. of cases 3-3	No. of transplanted cells	extinction No. of cells	encumbering %	extinction No. of cells	encumbering %	Date of treatment Oxygen content of the water	Date of the assessment
		Type of cell					
		H-59	H-59	LLT-HH	LLT-HH		
1. Control	10^3	0,932		0,629		---	6 th day
2. 40% OGV	10^3	0,822	11,8	0,222	64,7	1. 129,1% 4. 124,5% 5. 130,4%	6 th day
3. 80% OGV	10^3	0,513	44,9	0,082	86,8	1. 129,1% 4. 124,5% 5. 130,4%	6 th day
4. 20% OGV	10^3	0,289	69	0,067	90,46	1. 129,1% 4. 124,5% 5. 130,4%	6 th day

The FCS content of the nutritive solution was 10% initially, and then it was changed to 5% on the 3rd day

Experiment 2 The effect of OGV on the growth of tumour cells								
Treatments on the 1 st , 2 nd , 3 rd , 4th and 5th days, Assessment on the 7 th day								
Groups	No. of transplanted cells	extinction No. of cells	encumbering %	extinction No. of cells	encumbering %	Date of treatment	Date of the assessment	
3-3						Oxygen content of the water		
			Type of cells					
			H-59	H-59	LLT-HH	LLT-HH		
1. Control	10 ³	2,000		1,800	-	---	7 th day	
2. 40% OGV	10 ³	1,341	33%	0,366	81,4%	1.129,1% 4.124,5% 5.130,4% 6.116,5%	7 th day	
3. 80% OGV	10 ³	0,531	73,5%	0,00	100%	1.129,1% 4.124,5% 5.130,4% 6.116,5%	7 th day	
4. 20% OGV	10 ³	0,219	89,1%	0,00	100%	1.129,1% 4.124,5% 5.130,4% 6.116,5%	7 th day	

The FCS content of the nutritive solution was 10%, we changed it to 5% on the 3rd day

It is apparent from the 1st experiment that 3 and 4 OGV treatments decreased the tumour cell count significantly; it encumbered the growth of the tumour cells with about 90-100%.

Experiment 2.

In the second experiment we transplanted 10^3 tumour cells to a plate with 24 holes in a nutritive solution with an FCS content of 5%. We carried out the experiment – similarly to the second one – on the 2nd and 3rd days following the transplantation. So we started the treatments one day later than in the first experiment. We determined every time the oxygen content of the water and recorded it in the table.

We assessed the results of the experiment on the 6th and 7th days with the aid of the MTT assay.

Experiment 2 The effect of OGV on the growth of tumour cells (OGV = high oxygen content water, DV= distilled water)								
Treatments on the 2 nd and 3 rd days								
Groups	No. of transplanted cells	extinction No. of cells	encumberin g %	extinction No. of cells	encumberin g %	Date of treatment	Date of the assessment	
No. of cases						Oxygen content of the water		
3-3								
			Type of cells					
			H-59	H-59	LLT-HH	LLT-HH		
1. Control DV	10^3	0,244	-	0,317	-	---	6 th day	
2. 40% OGV	10^3	0,105	57%	0,140	56%	2.119,8% 3.113,7%	6 th day	
3. 80% OGV	10^3	0,102	58,2%	0,146	54%	2.119,8% 3.113,7%	6 th day	
4. 20% OGV	10^3	0,097	60,7%	0,165	48%	2.119,8% 3.113,7%	6 th day	
The FCS content of the solution was 5%, we started the treatments on the day following the transplantation								

The second experiment shows that even the two OGV treatments started on the second day prevented the growth of the tumour cells with about 50-60%.

Experiment 3.

In the third experiment we compared the effect of the oxygenated water (OGV) and the boiled oxygenated water (FOGV). We transplanted 10^3 tumour cells to a plate with 24 holes in a nutritive solution with an FCS content of 5%. We carried out the experiment similarly to the second one with the difference, that we carried out the experiment on the 2nd and 3rd days following the transplantation. So we started the treatments one day later than in the previous experiments. We determined every time the oxygen content of the water and recorded it in the table.

We assessed the results of the experiment on the 6th day with the aid of the MTT assay.

Experiment 2 The effect of OGV on the growth of tumour cells (OGV = high oxygen content water, DV= distilled water)					
Treatments on the 2 nd and 3 rd days					
Groups	No. of transplanted cells	extinction No. of cells	encumberin g %	Date of treatment Oxygen content of the water	Date of the assessment
No. of cases 6-6					
		LLT-HH	LLT-HH		
Control DV	10^3	0,440		---	6 th day
80% FOGV	10^3	0,456	0%		6 th day
80% OGV	10^3	0,316	28,2%	2.119,8% 3.113,7%	6 th day
20% OGV		0,290	33%	2.119,8% 3.113,7%	6 th day
40% OGV		0,157	79,1%	2.119,8% 3.113,7%	6 th day

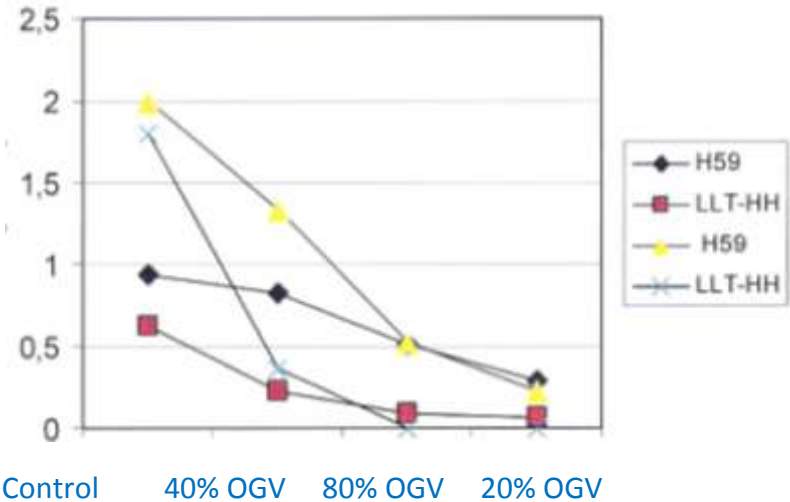
In the third experiment the OGV exerted encumbering effect to the tumour cells even during the first two treatments, while the FOGV did not exert any effect on the proliferation of the tumour cells.

To sum up the above we can conclude that the high oxygen content water decreases the tumour cell count in every cases.

If the oxygen content of the water is higher and the number of the treatments is bigger the preventive effect is stronger. (Experiment 1, Figure 1.2)

Figure 1. Encumbering effect of the high oxygen content water on the tumour cells in 4 treatments

The % of the oxygenated water in the nutritive solution after 3,4 treatments against the MTT extinction cell No.

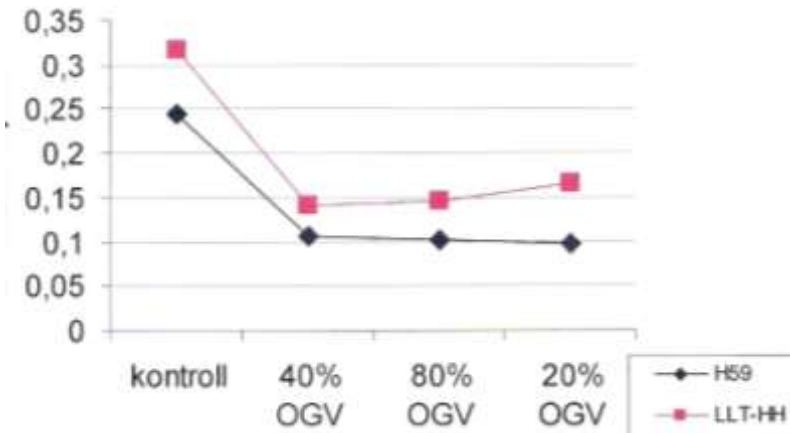


The preventive effect can be seen well with a microscope. (Picture 1)

Figure 1. The effect of the high oxygen content water on the growth of the tumour cells (OGV) (upper row control, 2 bottom rows treated)

Figure 2. The encumbering effect of the oxygenated water on the tumour cells

The % of the OGV in the nutritive solution after 2 treatments against the MTT extinction cell No.



The boiled water (FOGV) does not have an encumbering effect, so the preventive effect is in connection with the oxygen content of the water (experiment 3, figure 3).

The effect of FOGV and OGV on the tumour cells

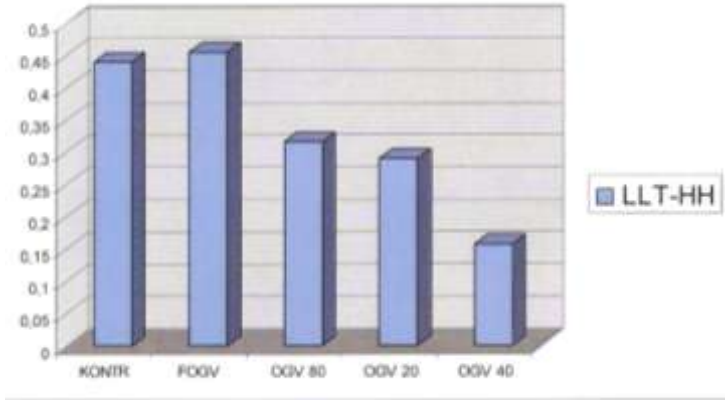
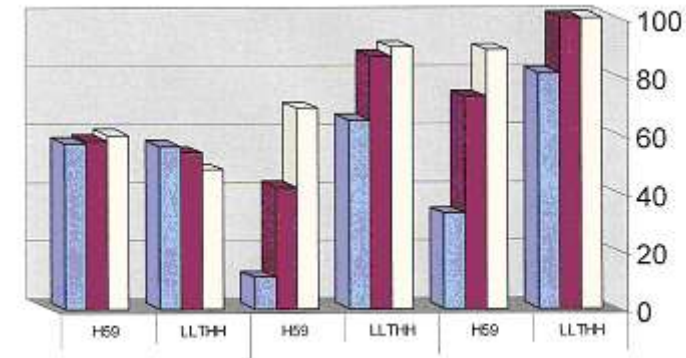


Figure 3. The effect of FOGV and OGV on the tumour cell growth

The level of the preventive effect is can be seen on figure 4.

Demonstration of the incumbering effect of OGV on the tumour cells in %



1 treatment 2 treatments 3 teatments

Figure 4. Demonstration of the incumbering effect of OGV on the tumour cells in terms of the number of treatments

Literature consulted

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Budapest, 21.12.2004.

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