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 $2x10^{-3}$ 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-dymethyl thiazol –2 –yl –2-5-diphenyltetrazolium bromide) assay. The measurement is based on the phenomenon that the 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 5^{th} and 7^{th} 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		---	6th day
2. 40% OGV	10^3	0,822	11,8	0,222	64,7	1. 129,1% 4. 124,5% 5. 130,4%	6th day
3. 80% OGV	10^3	0,513	44,9	0,082	86,8	1. 129,1% 4. 124,5% 5. 130,4%	6th day
4. 20% OGV	10^3	0,289	69	0,067	90,46	1. 129,1% 4. 124,5% 5. 130,4%	6th 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 1st, 2nd, 3rd, 4th and 5th days, Assessment on the 7th 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 cells				
			H-59	H-59	LLT-HH	LLT-HH	
1. Control	10^3	2,000		1,800	-	---	7th 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%	7th 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%	7th 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%	7th 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 2nd and 3rd days

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 cells				
			H-59	H-59	LLT-HH	LLT-HH	
1. Control DV	10^3	0,244	-	0,317	-	---	6th day
2. 40% OGV	10^3	0,105	57%	0,140	56%	2.119,8% 3.113,7%	6th day
3. 80% OGV	10^3	0,102	58,2%	0,146	54%	2.119,8% 3.113,7%	6th day
4. 20% OGV	10^3	0,097	60,7%	0,165	48%	2.119,8% 3.113,7%	6th 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 2^{nd} and 3^{rd} 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 6^{th} 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 cases 6-6	No. of transplanted cells	extinction No. of cells	encumbering %	Date of treatment Oxygen content of the water	Date of the assessment
		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

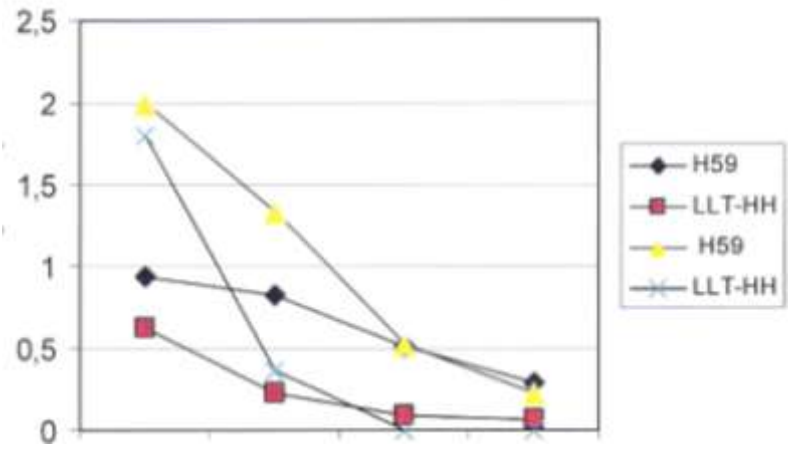
In the third experiment the OGV exerted encumbering effect to the tumour cells even during the first two treatments, while the FOGV did not exert any effect on the proliferation of the tumour cells.

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.



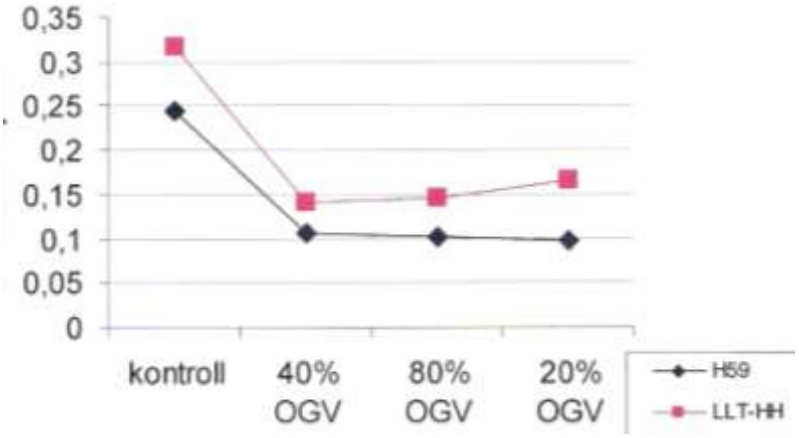
Control 40% OGV 80% OGV 20% OGV

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

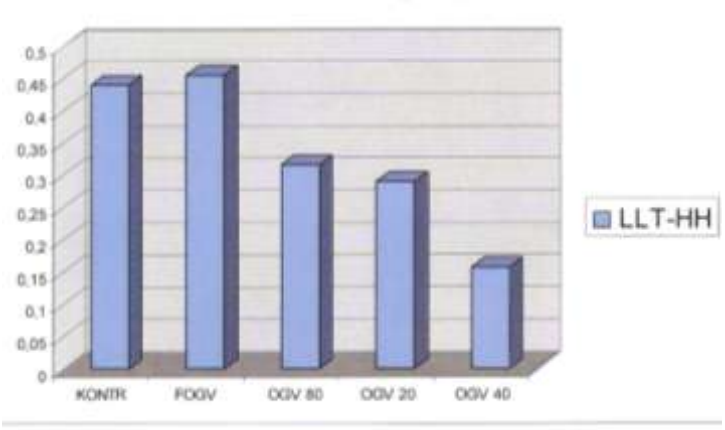
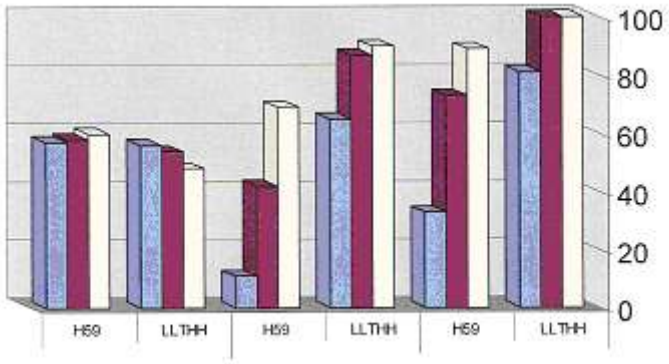


Figure 3. The effect of FOGV and OGV on the tumour cell growth

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

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Budapest, 21.12.2004.

dr Pál Katalin

candidate

2007 - Semmelweis / Changes of registered, psycho-physiological parameters by drinking „KAQUN”, water with high oxygen concentration

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The Psycho-physiological effects of the high OXYGEN content „KAQUN WATER" drinking cure and bath

1st part

Introduction

The results of earlier examinations suggested that the consumption of the high oxygen content kaqun water results in an increase in the oxygen saturation. On behalf of the Centrion Hungária Ltd. we have examined the change in the other physiological parameters we agreed on.

The aim of this study is to examine objectively the oxygen saturation, the reaction time, the exertion of forces, the blood pressure, the data can be derived from the ECG, the stress index and the standing stability

during the continuous consumption of the high oxygen content Kaqun water and before and after a simultaneous Kaqun bath.

We performed the measurements in the Kerepes Kaqun Gold Klub on 26th May 2007.

Methodology

The persons taking part in the measurement

The participants: 6 women and 4 men. Their average age is : 37.8 years.

The tools and instruments used

We measured the oxygen saturation with the „Oxycard" instrument being the property of the commissioner and manufactured by the Innomed Rt. (Budapest). This instrument beside the mentioned parameter can also display the pulse per minute count.

We registered the choice reaction time with the patented „Psycho 8" type differential psycho-physiological measuring instrument. We did the measurements on both left hand and right hand. We registered the individual averages, the deviations, and the „A" and „B" type mistakes. „A" type mistake occurs when the participant did not react to the stimulus, and „B" type is the mistake when the choice was incorrect.

The gripping force of the hand was measured with the „Psycho 8" measuring instrument with the aid of a special adapter. The screen showed the data in both numerical and diagram format. We measured the gripping force of both the left and right hand.

We registered the cardiologic data with the Vicargo instrument. The instrument is the property of the Energy Lab. Technology, Hamburg. The instrument provides the parameter of the state of the heart (scale of 0-5), the stress index (scale of 0-100 %), and the pulse per minute count. Besides these parameters it performs a complex calculation on the basis of

the digitalized data of the ECG. Among them there are the FFT analysis, the time of period histogram and the Poincaré diagram.

The measurement periods followed each other in case of each participant in 1,2 hours. (length of intervals)

We applied 2x20 stimuli in measuring the reaction time.

We adjusted the hand-gripping adapter to the size of the participants' hands in measuring the gripping force.

We did the Vicardio measurements with 4 electrodes, and we applied the Einthoven layout.

We examined the standing stability with the stabilometer instrument consisting of a force measuring platform, an amplifier, a micro computer and a Laptop.

We measured the blood pressure with the OMRON automatic instrument being the property of the commissioner.

The temperature of the Kaqun bath was 38^{o}C and the time interval was 50 minutes.

The target fluid intake was 5-7 dl during the measurement.

The abbreviations used in the tables:

O2 sat.	Oxygen saturation %
RT right	reaction time ms (with the right hand)
RT left	reaction time ms (with the left hand)
RT dev.	Deviation of the reaction time ms (st. dev.)
Force right	gripping hand force (with the right hand)
Force left	gripping hand force (with the left hand)
Cardio	the parameter of the sate of the heart on a scale of 0-5 (5 is the best)
Stress	stress index, on a scale of 0-100% (it is suitable under 35 %)
Pulse	the (average) pulse per minute count
Ro. I.	the measurement value of the standing stability in the Romberg test . the radius of the characteristic circle in mm, (the smaller the better value) the measurement takes place with open eyes
Ro. II.	the measurement value of the standing stability in the Romberg test . the radius of the characteristic circle in mm, (the smaller the better value) the measurement takes place with closed eyes.
Blood pressure	in this slot there are two values for the systole and diastole values.

Results

The results of the measurements can be seen in Tables 1.a / and b/. The data groups marked with the letters a/ and b/ summarizes the results of the two measurement cycles, and related to the time schedule of the measurement.

Discussion

The examination of the biological effect mechanism of the high oxygen content Kaqun water was not in the scope of this study.

The time consumption of the measurement of the high number of parameters lengthened the measurement cycle considerably, so it can be defended, that we in a few cases registered the results in the descending branch of the diagrams showing the effects of the Kaqun water.

We should note that the practice and the motivation can affect the results in certain parameters. In case of measuring the reaction time (1) practice, several repetition can improve the results. The operation of the answer button can be optimised. In case of the force measurement (2) the result also can be improved in a smaller degree with choosing the best way of holding. This can be achieved through several attempts. In both cases the degree of concentration is also an important factor.

The motivation of the participant in case of the standing stability measurement (3) also can play an important role. This effect, however, is small in extent. In order to eliminate these factors affecting the results in small extents of the first two measurement activities mentioned above (2,3) we made the participants perform enough practice exercises before the measurements.

In case of the Vicardio, the measurement of oxygen saturation and blood pressure these factors play hardly any role if the conditions of the measurement are prepared well before.

The parameter measuring the oxygen saturation after using Kaqun water was higher by an average of 1.2 %. Analysing the results of the participants one by one we see that after consumption 8 persons' results improved, one did not change and there was decrease in one case only. As during the measurement the level of oxygen saturation changes continuously, to assign the results to the actual level would be only possible with measuring the oxygen saturation continuously, but it was not feasible technically, as we had only one measurement instrument.

The average decrease of the reaction time (improvement) is 22 ms operating the answer button with the right hand after Kaqun water consumption. There was an improvement in case of 8 persons and there was decline only in 2 persons' results. When they operated the button with the left hand there was improvement in 6 cases and decline in 2 cases. The average improvement was 7.5 ms.

In case of analysing the force exertion we experienced an increase of 54 N with the right hand and 35 N with the left hand compared with the first control measurements.

As for the Vicardio results fatigue can only play a role. The value of the "heart state" parameter decreased by a small amount of 0.14. It is notable that the average stress decreased from 22,4% to 16.8%, so it improved. The same tendency can be traced in case of the pulse, the average pulse count decreased by 6 pulses per minute.

In the Romberg test we measured a 14% better result with open eyes, and with closed eyes the performance decreased by 7.8 %.

After the Kaqun treatment the blood pressure decreased by 2 %, although after the bath the reverse result would not be surprising either.

The measurement of several parameters was time consuming and seemingly it was tiring for some of the participants. The increase in the number of participants and the decrease in the number of parameters measured could improve the measurement results.

We can conclude that in the empirical measurements conducted we experienced favourable effects. The registered, mostly encouraging results can be the result of several factors. Among them there is the favourable effect of the high oxygen content Kaqun water by the increase in the oxygen saturation.

Budapest, 31st May 2007

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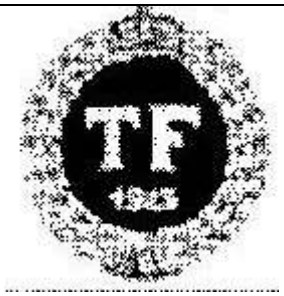
Faculty of Physical Education and Sport Sciences (TF)

44, Alkotás Str., Budapest, H-1123 Tel.: 487-92-00 Fax: 356-63-37		Biomechanics Department Head of department : Dr. Tihanyi József Doctor of the Hungarian Academy of Sciences

The Psycho-physiological effects of the high OXYGEN content „KAQUN WATER" drinking cure and bath

2nd part

Introduction

On behalf of the Centrion Hungária Ltd. we repeated our earlier measurements with other participants but the same method. We again measured the physiological parameters settled in our agreement.

The aim of this study is to examine objectively the oxygen saturation, the reaction time, the exertion of forces, the blood pressure, the data can be derived from the ECG, the stress index and the standing stability during the continuous consumption of the high oxygen content Kaqun water and before and after a simultaneous Kaqun bath.

We performed the measurements in the Rehabilitation Centre of the St. Stephen Hospital on 1st June 2007.

METHODOLOGY

The persons taking part in the measurement

The participants: 4 women and 6 men. Their average age is : 38.8 years.

The tools and instruments used

We measured the oxygen saturation with the „Oxycard" instrument being the property of the commissioner and manufactured by the Innomed Rt. (Budapest). This instrument beside the mentioned parameter can also display the pulse per minute count.

We registered the choice reaction time with the patented „Psycho 8" type differential psycho-physiological measuring instrument, after a suitably long practice. We did the measurements on both left hand and right hand. We registered the individual averages, the deviations, and the „A" and „B" type mistakes. „A" type mistake occurs when the participant did not react to the stimulus, and „B" type is the mistake when the choice was incorrect.

The gripping force of the hand was measured with the „Psycho 8" measuring instrument with the aid of a special adapter. The screen showed the data in both numerical and diagram format. We measured the gripping force of both the left and right hand.

We registered the cardiologic data with the Vicargo instrument. The instrument is manufactured by and the property of the Energy Lab. Technology, Hamburg. The instrument provides the parameter of the state of the heart (scale of 0-5), the stress index (scale of 0-100 %), and the pulse per minute count. Besides these parameters it performs a complex calculation on the basis of the digitalized data of the ECG. Among them there are the FFT analysis, the time of period histogram and the Poincaré diagram.

The measurement periods followed each other in case of each participant in 1,2 hours. (length of intervals)

We applied 2x20 stimuli in measuring the reaction time.

We adjusted the hand-gripping adapter to the size of the participants' hands in measuring the gripping force.

We did the Vicardio measurements with 4 electrodes, and we applied the Einthoven layout.

We examined the standing stability with the stabilometer instrument consisting of a force measuring platform, an amplifier, a micro computer and a Laptop.

We measured the blood pressure with the OMRON automatic instrument being the property of the commissioner.

The temperature of the Kaqun bath was 38^{o}C and the time interval was 50 minutes.

The target fluid intake was 5-7 dl during the measurement.

The abbreviations used in Table1:

O2 sat.	Oxygen saturation %
RT right	reaction time ms (with the right hand)
RT left	reaction time ms (with the left hand)
RT dev.	Deviation of the reaction time ms (st. dev.)
Force right	gripping hand force (with the right hand)
Force left	gripping hand force (with the left hand)
Cardio	the parameter of the sate of the heart on a scale of 0-5 (5 is the best)

Stress	stress index, on a scale of 0-100% (it is suitable under 35 %)
Pulse	the (average) pulse per minute count
Ro. I.	the measurement value of the standing stability in the Romberg test . the radius of the characteristic circle in mm, (the smaller the better value) the measurement takes place with open eyes
Ro. II.	the measurement value of the standing stability in the Romberg test . the radius of the characteristic circle in mm, (the smaller the better value) the measurement takes place with closed eyes.
Blood pressure	in this slot there are two values for the systole and diastole values.

RESULTS

The results of the measurements can be seen in Tables 1.a / and b/. The data groups marked with the letters a/ and b/ summarizes the results of the two measurement cycles, and related to the time schedule of the measurement.

DISCUSSION

The examination of the biological effect mechanism of the high oxygen content Kaqun water was not in the scope of this study.

The time consumption of the measurement of the high number of parameters lengthened the measurement cycle considerably, so it can be defended, that we in a few cases registered the results in the descending branch of the diagrams showing the effects of the Kaqun water.

We also considered in this measurement serial that the practice and the motivation can affect the performance in certain parameters. In case of measuring the reaction time (1) practice, several repetition can improve the results. The operation of the answer button can be optimised. Thus, we elongated the measurement cycle in a way that the faulty answers disappeared practically and the speed of reaction did not seem to change any more.

In case of the force measurement (2) the result also can be improved in a smaller degree with choosing the best way of holding. This can be achieved through several attempts before the measurement. In both cases the degree of concentration is also an important factor.

The motivation of the participant in case of the standing stability measurement (3) also can play an important role.

We paid attention to eliminate the factors affecting unfavourably the objectivity of the measurement regarding the above parameters 1,2 and 3.

In case of the Vicardio registration, the Oxycard measurement and the blood pressure the above mentioned disturbing factors play hardly any role if the conditions of the measurement are prepared well before.

The parameter measuring the oxygen saturation after using Kaqun water was higher by an average of 0.5 % at the time of the second measurement. Analysing the results of the participants one by one we see that after consumption 6 persons' results improved, two did not change and there was decrease in two cases only.

We must note here that the consumption because of the long cycles also was elongated in time in this measurement serial.

In the second measurement serial compared to the first, the average decrease of the reaction time (improvement) is 20 ms operating the answer button with the right hand after Kaqun water consumption. We must note here, that the result was a week before 22 ms, which is a very similar value. There was an improvement in case of 8 persons and there was decline only in 2 persons' results. When they operated the button with the left hand there was improvement in 7 cases and decline in 3 cases. The average improvement was 16.2 ms.

In case of analysing the force exertion compared to the results of the first measurement serial we experienced an increase of 19.4 N with the right hand and 20.4 N with the left hand.

As for the Vicardio results fatigue ccould only play a role. The value of the "heart state" parameter improved by a small amount of 0.09. The average stress index decreased from 23,8% to 19%, so it improved, similarly to the participants' of the first serial. The same tendency can be traced in case of the pulse, although the decrease of the average pulse count was minimal.

In the Romberg test we measured a 4.5% worse result with open eyes, and with closed eyes the performance decreased by 7.8 %. As we did this test last, the participants said after several hors of active co-operation they were very tired.

After the Kaqun treatment the blood pressure decreased by 5.7 % (systole) and increased by 2.3 % (diastole)

The measurement of several parameters was time consuming and seemingly it was tiring for some of the participants. The increase in the number of participants and the decrease in the number of parameters measured could improve the measurement results.

We can conclude that in the empirical measurements conducted we experienced favourable effects.

As a matter of fact, the results were repeated, they confirmed the improvement in terms of the parameters regarding which we expected positive effects. The registered, mostly encouraging results can be the result of several factors. Among them there is the favourable effect of the high oxygen content Kaqun water by the increase in the oxygen saturation.

Budapest, 4th June 2007

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2009 - The effect of KAQUN-water on the immune parameters of healthy volunteers /NICS/

NATIONAL INSTITUTE OF CHEMICAL SAFETY

Report

The effect of KAQUN-water on the immune parameters of healthy volunteers

Budapest

2009

Report
The effect of KAQUN-water on the immune parameters of healthy volunteers

Antecedents

KAQUN HUNGÁRIA Ltd. (2144 Kerepes, Szabadság út 102), as Client has contracted the National Institute of Chemical Safety/NICS) (1096 Budapest, Nagyvárad tér 2.) as contractor in contract no. GOKBI-360/2009 to test the immune effects of KAQUN water = Q voda in healthy volunteers at the Department of Cytogenetics and Immunology of NICS. Kaqun is a special water with high oxygen content, suitable for use in the form of drinking water or bathing. In our study we examined the effect of 21 days of bathing and drinking on the immune parameters of healthy volunteers. The end points measured were: qualitative and quantitative blood counts, the ratio of lymphocyte populations, lymphocyte activation and the oxidative burst of neutrophil granulocytes. The measurements were carried out on the first day before the start of the treatment (0 point) and on the 8^{th}, 15^{th} and 21^{st} days.

The theoretical basis of immunology tests

Immune-toxicology examines the damaging/modifying effects caused by exposure at the workplace, environment or therapy on the immune system. Its task is to detect and assess the modifying factors affecting the immune system especially from the aspect of their effect on human health. An immune response may be elicited when the immune system is the passive target of a chemical agent or when the chemical, as an antigen, triggers a specific response. In consequence of the complexity of the immune system the chemical agents have a broad target of attack. They can affect the development, maturation, division, differentiation and function of cells, or modify the regulation of the immune system.

The immunology tests were carried out on peripheral blood samples. Blood cells consist of *red blood cells* (erythrocytes), *white blood cells* (leukocytes) and *platelets* (thrombocytes). The volume ratio of blood cells in the blood is characterized by the *hematocrit* value.

Erythrocytes are formed in the bone marrow, their development takes about 4 -5 days, while their nucleic acid content gradually degrades, and mature red blood cells do not have a nucleus. The blood of an adult contains an average of $4.5x10^{12}$ / l erythrocytes (for women the average is about $4,5x10^{12}$ / l, for men it is somewhat higher, $5x10^{12}$ / l). During maturation erythrocytes synthesize hemoglobin molecules, which are able to carry oxygen.

The average life-span of erythrocytes is 120 days, and they are degraded in the spleen and liver. More than 99% of the blood cells are erythrocytes.

White blood cells have an important role in the defence mechanisms of the body. Blood contains an average of $9x10^9$ / l white blood cells, but 4-10 x 10^9 / l is also within the normal range. There are 3 main types of leukocytes: *granulocytes, monocytes and lymphocytes*. 50-75 % of leukocytes of a healthy person are granulocytes, 20-45 % are lymphocytes and 2-9 % are monocytes.

The horseshoe shaped nucleus of immature granulocytes becomes lobed as they mature. Another characteristic feature is the presence of large quantities of granules in the cytoplasm – the biologically active material stored within them has a very important role in the development of inflammation and allergic reactions. The *neutrophil, basophil* and *eosinophil granulocytes* can be distinguished on the basis of their histological staining properties. Most of the granulocytes are *neutrophils* (3-6 x 10^9 / l). Since their half life in the circulation is short, (generally ~6 hours), they are produced in large quantities every day. They are the basis of cellular protection against infection, and can enter the tissues in large quantities. In the course of bacterial or fungal infection the neutrophil granulocytes phagocytose and destroy the pathogens. The intracellular killing of pathogens is achieved by oxygen-independent enzymes (lysosomal elastase, lysosime) and oxygen-dependent enzymatic systems (principally NADPH-oxidase). The activated phagocytic cells produce antimicrobial reactive radicals, so called reactive oxygen intermediates (ROI) in a reaction named oxidative burst.

Under normal conditions the number of *eosinophils* is far less in the circulation (1.5-3.0 x 10^8 /l); they are mostly found in the mucous membranes of the respiratory, urinary, and intestinal tract participating in the protection against parasites. The number of eosinophils circulating in the vascular system increases in the case of allergic reactions. The *basophils*, similarly to mast cells, contain heparin, histamine and other inflammatory mediators in their granules. Their number is low (<1 x 10^8 /l), they are important because they mediate immediate type hypersensitivity and anaphylactic reactions.

Normally the *lymphocyte* count is in the range of 1.5-3.5 x 10^9 /l, and their importance lies in mediating the adaptive immune response. They are relatively small cells, their round shaped nucleus fills the cytoplasm almost completely. Lymphocytes are classified into 3 main groups: *T lymphocytes* are responsible for the so called cellular immune response, while *B lymphocytes* are responsible for the humoral immune response, and the production of antibodies. The *NK cells* kill virus infected or cancerous cells.

Monocytes make up about 2-9 % of the white blood cells (1-8 x 10^8 /l), their nucleus is large, kidney or bean shaped. They originate from the bone marrow, they then enter the circulation where they spend about 72 hours, and then pass through the blood vessel wall and change into *tissue macrophages*. Their activation is initiated by lymphokines secreted by T lymphocytes, and as a result they become able to phagocytose foreign matter such as bacteria, and to release a number of inflammatory mediators (e.g. prostaglandin-E).

Platelets (*thrombocytes)* are cytoplasmic fragments of megakaryocytes surrounded by a cell-membrane; they do not have a nucleus. Their size is approximately 2-5 □m. When leaving the bloodstream or encountering damaged endothelial walls they are activated and play an

important role in blood coagulation. The average thrombocyte count is 3 x 10^{11} /l, but a value in the range of 1.5–4.0 x 10^{11} /l is normal.

The immune system has an evolutionarily old, non-specific arm which reacts immediately upon infection. Its most important elements are macrophages, granulocytes, NK cells and the complement system. Macrophages and *granulocytes* have an important role in the phagocytosis of pathogens and foreign particles, while *NK cells* destroy virus-infected and cancerous cells. The pathogen organisms that enter the body first meet this so-called innate immune system. Built on this, is the specific (antigen specific) adaptive immune system, which reacts slowly (in days) when first meeting the antigen, but has an immunologic memory; therefore it works fast and efficiently in the case of a second infection. T and B lymphocytes are the cells of the adaptive immune system. During the adaptive immune response *cytotoxic T (Tc)* cells are generated which are able to destroy the pathogens directly (cellular immune response), and *B lymphocytes*, which produce antibodies (humoral immune reaction). The presence of *helper T lymphocytes (Th)* is essential for the division and differentiation of the T and B cells. Cell-cell interactions and cytokines produced by leukocytes have an important role in the regulation of the immune response.

A number of molecules, "markers" appear on the surface of lymphocytes and with their help the lymphocyte populations can be distinguished from each other. These markers have been classified into groups, and each marker has been given a CD (Cluster of Differentiation) number. The basic lymphocyte populations (T, B, NK cells) can be defined with cell markers: *T lymphocytes* express CD3 (CD3+ cells), *helper T cells* also express CD4 (CD4+/CD3+ cells), *cytotoxic T cells* express CD8 besides CD3 (CD8+/CD3+ cells). Immature T cells express both the CD4, and the CD8 molecules (CD4+/CD8+ cells). *B lymphocytes* can be characterized by the CD19 cell surface antigen (CD19+cells). *NK cells* have CD56 surface molecules, but do not express CD3, therefore they are characterized as CD56+/CD3- cells. CD25 (IL-2 receptor) and CD71 (transferrin receptor) surface antigens cannot be detected on resting lymphocytes, they are expressed when the lymphocytes are activated (e.g. by an antigen). Therefore these surface molecules can be used to detect the activation of lymphocytes.

Immunotoxic materials can affect different immune parameters; therefore we have adjusted our measurements to characterize different functions. This is important, because the change in one parameter or another is not suitable to characterize the general condition of the immune system, conclusions can only be drawn from changes in the data pattern. We characterized the immune status of the studied subjects by measuring characteristics of white blood cells gained from peripheral blood. Qualitative and quantitative blood count was determined, and immune phenotyping was used to determine lymphocyte subpopulations and the CD25 (IL-2R) and CD71 (transferrin receptor) activation antigens expressed on lymphocytes with the aid of monoclonal antibodies produced against cell surface molecules.

Innate immunity was characterized with the help of a functional test: the killing capacity of white blood cells was determined by measuring the production of reactive oxygen intermediates (ROI) of granulocytes.

Test procedure

Selection of healthy volunteers

The selection of 30 healthy volunteers (15 women, 15 men) was carried out by KAQUN HUNGÁRIA Kft. Exclusion criteria in this study were: acute or chronic illness, infection, the use of any kind of drugs, and smoking, because these could affect immune parameters.
The participants were informed about the purpose and the course of the study, and they signed a *Declaration of Agreement* confirming that they had received information about the study and that their participation was voluntary.

Duration of the study and the procedure:

The examined persons participated in a 21 day bathing and water drinking treatment. The participants bathed once a day in the morning in individual bathtubs filled with 37 ^{o}C water containing stable oxygen, for a maximum of 50 minutes per occasion. The water drinking cure consisted of drinking 1.5 liter Kaqun drinking water every day in parallel with the baths. The bathing cure followed the standards established in the Kaqun Health Program Service.

The 21 days Kaqun bathing and the parallel water drinking treatment was divided into 4 groups, because only 7-8 persons could be examined in a single day. All four groups started on the first week, the first on Monday, the second on Tuesday, the third on Wednesday and the fourth on Thursday. The participants of the first group were always examined on Monday, the second on Tuesday and so on, see table below.

	1st week	2nd week	3^{d} week	4th week
Monday 1st group	day 1 blood sampling before treatment	day 8 blood sampling after treatment	day 15 blood sampling after treatment	day 21 blood sampling after treatment
Tuesday 2nd group	day 1 blood sampling before treatment	day 8 blood sampling after treatment	day 15 blood sampling after treatment	day 21 blood sampling after treatment
Wednesday 3^{d} group	day 1 blood sampling before treatment	day 8 blood sampling after treatment	day 15 blood sampling after treatment	day 21 blood sampling after treatment
Thursday 4th group	day 1 blood sampling before treatment	day 8 blood sampling after treatment	day 15 blood sampling after treatment	day 21 blood sampling after treatment

Methods:

Blood sampling:
Blood sampling at the site: day 1 before the bath, (0-point), then on days 8, 15, and 21 after the bath during the same part of the day. The blood samples were taken from the cubital vein of the examined persons in sitting position, under sterile conditions with venipunction. Standard 3 ml sterile vacuum blood sampling tubes containing anti-coagulant were used for blood sampling. One 3 ml tube with EDTA anti-coagulant for determining the qualitative and quantitative blood count, one 3 ml tube with heparin for the immunology tests. The blood samples were given unique identifiers marked on the blood sampling tubes.

The following tests were carried out on the blood samples:

1) Qualitative and quantitative blood count

The qualitative and quantitative blood count was carried out with an automated analyser in the blood sampling laboratory of OMFI (Bp. IX. Nagyvárad tér 2.).

Determined parameters:

- WBC leukocyte count,
- abs LY, abs MO, abs NEUTR, abs EO: the absolute number of lymphocytes, monocytes, neutrophil- and eosinophil granulocytes
- LY %, MO %, NEUTR %, EO %, BA %: percentile distribution of lymphocytes, monocytes, neutrophil- eosinophil- and basophil granulocytes
- RBC red blood cell count,
- Hb concentration of hemoglobin in the blood,
- HTK hematocrit,
- MCV mean cell volume,
- MCHC mean corpuscular hemoglobin concentration,
- RDW-CV red blood cell distribution width
- MCH mean cell hemoglobin,
- Thrombocyte count

2) Determination of immune parameters

Method:

The subpopulations and activation of circulating lymphocytes were determined by immune phenotyping, using flow cytometry. Heparinized whole blood was used for the measurement. The surface markers of peripheral lymphocytes were measured with fluorescent labelled monoclonal antibodies in a flow cytofluorimeter. The surface antigens

examined were: CD3 (T-cell receptor), CD4 and CD8 (T-cell co-receptors), CD19 (B-cell co-receptor), CD25 (interleukin-2 receptor), CD45 (protein-tirosine-phosphatase, pan leukocyte marker), CD56 (neural cell adhesion molecule, NK-cell marker), CD71 (transferrin receptor). Using 3 and 4 colour staining the following antibody combinations were used: (1) CD25-FITC / CD8-PE / CD3-PerCP / CD4-APC; (2) CD56-FITC / CD3-PerCP / CD45-APC; and (3) CD71-FITC / CD3-PerCP / CD19-APC. Standard forward and side scatter gating combined with CD45 was used to separate leukocyte populations and to set the lymphocyte gate. The lymphocyte subpopulations of the donors (T lymphocyte, helper T, cytotoxic T, B lymphocyte and NK-cell) were determined with the aid of cell markers. CD25 and CD71 surface antigens were used to determine the activation of lymphocytes.

Determined parameters:

- Ly, Mo, Neu, Eos: percentage of lymphocytes, monocytes, neutrophil- and eosinophil granulocytes

- Total T, T helper, T cytotoxic, Immature T, B cell, NK-cell: percentage of T lymphocytes, cytotoxic and helper T lymphocytes, immature T lymphocytes, B lymphocytes and NK-cells within lymphocytes

- Th/Tc: The ratio of helper and cytotoxic T lymphocytes

- Activated T: percentage of CD25 (IL-2 receptor) activation antigen carrying T cells within the T cells
  - Activated Th: percentage of CD25 activation antigen molecule carrying helper T cells within the helper T cells
  - Activated Tc: percentage of CD25 activation antigen expressing cytotoxic T lymphocytes within the cytotoxic T lymphocytes
  - CD71 positive T: percentage of CD71 (transferrin receptor) molecule carrying T cells within the T cells
  - CD71 positive B: percentage of CD71 (transferrin receptor) molecule carrying B cells within the B cells

3) Oxidative burst of neutrophil granulocytes

The production of reactive oxygen intermediates (ROI) which is directly proportional with the killing potential of white blood cells was measured with the aid of Bursttest (Phagoburst®) kit. Neutrophil granulocytes respond to activation by producing reactive oxygen intermediates, which oxidize the fluorogenic substrate. The quantity of oxidized substrate is proportional to the production of reactive oxygen radicals. Heparinized whole blood was used, and the measurement was carried out on a flow cytometer. We measured the quantity of oxidized substrate in the control and the stimulated samples, and determined the percentage of ROI producing cells. The activation stimuli: 1) fMLP chemotactic peptide (weak stimulus). 2) E. coli opsonized with antibody, which stimulates through the Fc receptors that recognize the constant part of the antibody (particulate stimulus) 3) PMA (phorbol-myristil-acetate), which transports signals through protein kinase C (strong stimulus)

Determined parameters:

Production of reactive oxygen intermediates (ROI)

Control, fMLP, E. coli, PMA: ROI production in unstimulated samples, and samples stimulated with fMLP, E. coli, and PMA

Percent of ROI producing cells
Control, fMLP, E. coli, PMA: Percent of ROI producing cells in unstimulated samples, and samples stimulated with fMLP, E. coli, and PMA

Statistical analysis:

Student's paired-t test was used for the group level statistical evaluation of the results, the level of significance was set at $p<0.05$.

Results and conclusions

1) Qualitative and quantitative blood count

The group results of qualitative and quantitative blood counts are shown in *table 1,* the individual results in *table 2.* No significant change was observed for the group average of white blood cell count in any of the groups. Individually both increased and decreased leukocyte counts could be observed during the three weeks of the study. No change was observed for the group average of lymphocyte counts. On the other hand a statistically significant decrease was observed in the group average of monocyte counts during the treatment in all three groups. In men the count decreased after the first and second week of treatment, while the change was not significant after the third week compared to the 0 point. At the individual level the monocyte count does not change or a slight decrease can be observed. In men the group average of neutrophil granulocyte count increases after the second and third week of treatment. At the individual level generally an increase can be detected, but in a few cases a reduction was observed during the three weeks of the study. The eosinophil count decreased for the whole group by the second and third week; in the case of men the reduction was present already after the first week. There was no significant change in the group average for women. Individually no change could be observed above the uncertainty of the measurement.

The percentage of white blood cells shows a similar change to that of the absolute numbers. The percent of monocytes decreased at the group level for all three groups already after the first week of treatment. Further change was not observed. The percent of neurophils increased for the whole group and for men after the second week of treatment, the percent of eosinophils decreased in the whole group and in men already after the first week of the treatment.

At the group level there were no changes in the red blood cell count and hemoglobin content. After the second week of the treatment a slight decrease in hemocrit was observed for the whole group. The average volume of erythrocytes (MCV) showed a very slight decrease by the second week, therefore the hemoglobin concentration for one erythrocyte (MCH) and the average hemoglobin concentration of the erythrocytes (MCHC) increased to a small extent.

A statistically significant increase in the group average of thrombocyte count was observed after two and three weeks of treatment both for the whole group and in men. Examining the individual results, the subjects usually did not show large changes in the thrombocyte count, and the thrombocyte count always remained within the normal range.

Biologically significant change was not observed in the qualitative and quantitative blood count either at group or individual level.

2) Determination of immune parameters

The measurements carried out with the flow cytometer produced very similar results to those carried out with the automated analyser regarding the percentile distribution of lymphocytes, monocytes, neutrophil- and eosinophil granulocytes. This can be considered as the internal control of the measurements.

The group averages of immune parameters are shown in *table 3,* the individual results in *table 4.* The percentage of monocytes decreases at group level for the whole group and for men by the second week of the treatment. At the same time the percentage of neutrophil granulocytes increases at group level for the whole group and for men by the second week of the treatment. The percent of eosinophils decreases in the whole group from the first week of the treatment, and in the case of men by the second week of the treatment the decrease is significant. In women the above parameters do not change significantly. In the course of the treatment the ratio of leukocytes changes statistically, which could be indicative, but the changes are so small that probably no physiological importance can be attached to them.

No significant changes were observed in the percentage of total T cells, helper T cells, immature T cells and B lymphocytes. The ratio of helper and cytotoxic cells did not change (Th/Tc) either. In the case of men the ratio of cytotoxic T cells showed a small, but significant reduction after the third week of the treatment. The percentage of NK-cells increased significantly after the second week of the treatment both for the whole group and for women. In men an increase was observed, but due to the large deviations in individual results, the change was not significant statistically. Individually, in general either there was no change or an increase was observed during the three weeks of the study. Although the ratio of cytotoxic T lymphocytes showed a significant decrease, at the individual level the changes were so small, that a physiological effect cannot be expected. Relatively bigger changes (increase) were observed in the ratio of NK-cells at the individual level, compared to the 0 point, which may have a functional impact: more NK cells are available to kill virally infected or cancerous cells.

The percentage of activated (CD25+) T lymphocytes increased by the second and third week in the whole group and in men. At the individual level there is either no change or an increase can be observed, but in a few cases the percentage of activated T cells decreased in the three weeks of the study. The percentage of activated (CD25+) helper T cells increased for the whole group by the second week of the treatment. In general individually there is either no change or an increase can be observed. The percentage of activated (CD25+) cytotoxic T cells increased significantly after the third week in the case of men. The increase in the expression of the CD25 cell surface molecule indicates the activation of T lymphocytes. These results indicate the intensification of the cellular immune response.

The percentage of transferrin receptor positive (CD71+) T lymphocytes did not change during the treatment. The percentage of B lymphocytes expressing transferrin receptors (CD71+) decreased significantly by the second week in the whole group, and by the third week this value returned to the original level. The individual data show such a large

distribution both individually and intra-individually that a biologically relevant conclusion cannot be drawn from these data.

Among the examined persons, there was a man whose percentage of B lymphocytes was well below the reference value. The reference range for B lymphocytes is 7.0-23%. The B cell percentage of the person indicated as Q3,Q33,Q63,Q93 (Gábor Rabb) was between 0.3-0.9% during the period of the study. The white blood cell count and the absolute lymphocyte count did not decrease, but the percentage lymphocytes was low measured with both test methods, and the percent of B lymphocytes was extremely low. The B cell count (data calculated with the aid of the absolute number of lymphocytes and the percentage of B cells) was at least one order of magnitude less than in the case of the other subjects. His data were not included in the statistical analysis of immune parameters, as in our opinion they would have falsified the data.
On the 15th of June 2009 the blood sample of the person coded Q35 (István Berei) deviated to such an extent from the values measured during the three other occasions regarding certain parameters (percentage of lymphocytes measured both with the automated instrument and flow cytometer, percentage of helper T and NK-cells) that his data measured on 15.06.2009 were omitted from the group level evaluation of the immune parameters.

3) Killing capacity of neutrophil granulocytes (production of reactive oxygen intermediates-oxidative burst)

The group averages for the production of reactive oxygen intermediates of neutrophil granulocytes are shown in *table 5,* the individual results in *table 6.* The reactive oxygen intermediate production (ROI) of neutrophil granulocytes increased significantly in all three groups from the first week of the treatment in the fMLP and PMA stimulated samples, and from the second week in the samples stimulated with E. coli. Individually, in general an increase was observed in ROI production, though in the samples stimulated with E. coli and PMA a decrease relative to the 0 point was observed for certain individuals after the first week.

The percentage of ROI producing cells increased significantly in all three groups from the first week and this is also true at the individual level.

The increase in ROI production, and the fact that more cells respond to stimulation, result in the increased killing potential (bactericidal effect) of neurophil granulocytes.

Summary

1. No biologically significant changes were observed in the qualitative and quantitative blood count either at group level or individual level during the 21 days of Kaqun treatment.

2. The percentage of NK-cells showed a statistically significant increase, and the individual changes (increase) relative to the 0 point were bigger, which may have a functional impact, namely that more NK cells are available to kill virus infected and cancerous cells.

3. A non-specific activation of T lymphocytes (indicated by the increase in the expression of the CD25 cell surface antigen) could be detected, presumably caused by the Kaqun treatment, indicating the increased activity of the cellular immune response.

4. Characteristically the value of several parameters changed significantly by the second week of treatment and during the third week the value of the parameter remained at the same level, or the change levelled to its original value (percent of neurophils, monocytes, activated (CD25+) T cells, activated (CD25+) helper T cells and CD71+ B cells). This suggests that two weeks treatment is the most effective for the change in immune parameters and after that the reaction of the body to the treatment decreases, that is, the effect cannot be boosted.

5. The increase of the production of reactive oxygen intermediates both at group level and at the level of the individuals results in the intensification of the killing potential of neurophil granulocytes.

28th of September 2009

Dr. Anna Biró

Head of department

2010 - Citotoxicity examination of Kaqun water in HepG2 cells /NICS/

NATIONAL INSTITUTE OF CHEMICAL SAFETY

DEPARTMENT OF RESEARCH FOR CHEMICAL SAFETY

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Closing Report

Number of examination: 02-CTOX-10

Citotoxicity examination of Kaqun water in HepG2 cells

2010

Budapest

Citotoxicity examination of Kaqun water in HepG2 cells

Responsible Persons

	Signature	Date
Principal investigator	(illegible signature) Dr. Zsuzsanna Kocsis Biologist	20.12.2010
Department Head of OKBI-KBKF-MSBO	(illegible signature) Dr. Zoltán Macsek Ph.D. Biologist	20.12.2010
Department Head of OKBI-KBKF	(illegible signature) Dr. Jenő Major Ph.D. Biologist	20.12.2010
Director General of OKBI	(illegible signature) Dr. Imre Bordás Ph.D. Chief Physician	20.12.2010
Head of Quality Control Group of OKBI-KBKF	(illegible signature) Dr. Márta Kovács Pharmacist	20.12.2010

1. PRINCIPAL INVESTIGATOR'S DECLARATION

I the undersigned hereby declare that the toxicity examination titled Citotoxicity examination of Kaqun water in HepG2 cells (with examination number: 02-CTOX-10) was carried out in compliance with the regulations of OECD Principles of Good Laboratory Practice (ENV/MC/CHEM(98)17) at the Molecular and Cell Biological Department of the Department of Research for Chemical Safety of the National Institute of Chemical Safety (OKBI).

The examination was carried out based on the decrees titled Biological evaluation of medical devices Part 5: Tests for citotoxicity: *in vitro* methods (ISO 10993-5: 1992), MSZ EN 30993-5:1998; Biological evaluation of medical devices Part 12: Sample preparation and reference materials (ISO 10993-12: 2007), MSZ EN 10993-12:2008.

The examination was carried out according to Standard Operations Regulations of OKBI-KBKF-MSBO.

The Closing Report is based on correct examination data and the obtained results are in compliance with the content of the Closing Report.

Budapest, 20/12/2010

(illegible signature)

Dr. Zsuzsanna Kocsis

Principal Investigator

QUALITY ASSURANCE DECLARATION

Title of examination: Citotoxicity examination of Kaqun water in HepG2 cells

Number of examination: 02-CTOX-10

The examination took place observing the (ENV/MC/CHEM(98)17) Guidelines of OECD and no. 9/2001(III:30)EüM-FVM Joint Decree of the Ministry of Health and Ministry of Agriculture and Rural Development on "implementing and checking good laboratory practice".

The examination and Closing Report were audited by the Quality Control Group of OKBI-KBKF. The data published in the Closing Report as well as the methods and procedures applied in the examination reflect the raw data.

Dates of checking	Examination phases	Report dates	
		Principal Investigator	GLP management
04/11/2010	Draft examination plan 1	04/11/2010	04/11/2010
04/11/2010	Final examination plan	04/11/2010	-
10/11/2010	Treatment	10/11/2010	-
12/11/2010	Measuring optical density	12/11/2010	-
17-20/12/2010	Draft Closing Report 1	20/12/2010	20/12/2010
12/10/2010	Closing Report	20/12/2010	-

Budapest, 20/12/2010

(illegible signature)

Dr. Márta Kovács

Head of Quality Control Group

2. SUMMARY

Title of examination:	Citotoxicity examination of Kaqun water in HepG2 cells
Examination material:	Kaqun water for bathing
Examination concentrations:	Without dilution
Examined parameter:	Citotoxicity
Method:	MTT assay
Exposition time:	24 hours
Result	negative

Result of the citotoxicity examination

	Measurement results		Paint-reduction %	Evaluation
	Average	Standard deviation		
Positive control	0.047	0.005	27.16	Positive
DMEM with Kaqun water	0.189	0.024	109.2	Negative
DMEM with ultra-pure water	0.185	0.021	106.9	Negative
DMEM	0.173	0.031	100	Negative

3. Summary

In the given experimental conditions Kaqun water did not reduce the number of viable cells compared to the untreated control group.

Kaqun water does not have any citotoxic effect.

4. GENERAL INFORMATION

4.1. Title of examination

Citotoxicity examination of Kaqun water in HepG2 cells

4.2. Aim of examination

The aim of the examination is to assess the citotoxicity causing effect of Kaqun water.

4.3. Method of examination

The citotoxicity examination was carried out in compliance with the standards titled Biological evaluation of medical devices Part 5: Tests for citotoxicity: *in vitro* methods (ISO 10993-5: 1992), MSZ EN 30993-5:1998; Biological evaluation of medical devices Part 12: Sample preparation and reference materials (ISO 10993-12: 2007), MSZ EN 10993-12:2008.

The examination took place observing the regulations of no. 9/2001(III:30)EüM-FVM Joint Decree of the Ministry of Health and Ministry of Agriculture and Rural Development on "implementing and checking good laboratory practice", of OECD Principles of Good Laboratory Practice (ENV/MC/CHEM(98)17), and of OECD The Application of the Principles of GLP to the in vitro Studies (ENV/JM/Mono(2004)26).

4.4. Place of examination

National Institute of Chemical Safety

Department of Research for Chemical Safety Molecular and Cell biological Department

1096 Budapest, Gyáli út 2-6.

4.5. Sponsor

KAQUN HUNGÁRIA Kereskedelmi Kft.

2144 Kerepes, szabadság út 102.

Authorised representative: Dr. Gyula Sebestyén, Scientific Counsellor

Semmelveis Medical University

1097 Budapest, Nagyvárad tér 2.

5. EXAMINATION AND CONTROL MATERIALS

5.1. Chemical and physical properties of the examination material

Name:	Kaqun water, for bathing
Manufacturer:	Kaqun Hungária Kft.
Delivered quantity:	2 x 1.5 l
Manufacturing number:	25/10/2010

CAS number: -

Number of analytical certificate:	Kerepes (2010/K/2192)
Number of microbiological inspection:	1-1298-2010
Colour:	water clear, colourless
Smell:	without smell
Storage conditions:	at room temperature
Safety regulations:	-
Expiry date:	08/10/2011

5.1.1. Stability examination

No stability examination was carried out for Kaqun water.

5.2. Control materials and solvent

5.2.1. Culture liquid

Name: Dulbecco’s Medium W/Pyruvate powder

Manufacturer: Gibco Invitrogen Corporation

Manufacturing number: 757533

Storage conditions: 2-8°C

Safety regulations:-

Expiry date: 30/04/2011

Name: DMEM, Dulbecco's Modified Eagly Medium 1X

Manufacturer: Gibco Invitrogen Corporation

Manufacturing number: 712334

Storage conditions: 2-8°C

Safety regulations:-

Expiry date: 31/12/2010

5.2.2. Positive control

Name: Dimethyl sulfoxide

Manufacturer: Sigma-Aldrich Kft.

Manufacturing number: BCBB 0540

CAS number: [67-68-5]

Storage conditions: at room temperature

Safety regulations: use protective gloves and glasses

Expiry date: 30/03/2014

5.3. Other materials used for the examination

5.3.1. Penicillin-streptomycin solution

Name: Penicillin Streptomycin (100x)

Manufacturer: PPA Laboratories GmbH

Manufacturing number: P01009-1954

Storage conditions: below -15°C

Safety regulations: use protective gloves

Expiry date: 31/08/2011

5.3.2. Serum

Name: Foetal beef serum (FBS EU Approved origin)

Manufacturer: Gibco Invitrogen Corporation

Manufacturing number: 41Q8095F

CAS number:-

Storage conditions: between -5 and -20°C

Safety regulations: use protective gloves

Expiry date: 31/05/2014

5.3.3. Trypsin solution

Name: Trypsin-EDTA (10X)

Manufacturer: Gibco Invitrogen Corporation

Manufacturing number: 695604

CAS number:-

Storage conditions: between -5 and -20°C

Safety regulations: use protective gloves

Expiry date: 30/04/2011

5.3.4. PBS solution

Name: PBS pH 7,4 W/O CAMG USA

Manufacturer: Gibco Invitrogen Corporation

Manufacturing number: 779745

CAS number:-

Storage conditions: between 15 and 30°C

Safety regulations:-

Expiry date: 31/05/2012

5.3.5. Isopropyl-alcohol

Manufacturer: Sigma-Aldrich Kft.

Manufacturing number: 078K0666

CAS number:[67-63-0]

Storage conditions: between 15 and 30°C, under nitrogen protective gas

Safety regulations: use protective gloves

Expiry date: 30/06/2011

5.3.6. MTT paint

Name: Thiazolyl Blue Tetrazolium Bromide

Manufacturer: Sigma-Aldrich Kft.

Manufacturing number: MKBC3383

CAS number: [298-93-1]

Storage conditions: between 2 and 8°C

Safety regulations: use protective gloves

Expiry date: 31/10/2012

5.3.7. Sodium-hydrogen-carbonate

Manufacturer: Sigma-Aldrich Kft.

Manufacturing number: BCBB 8363

CAS number: [144-55-8]

Storage conditions: between 15 and 30°C

Safety regulations:-

Expiry date: 28/02/2015

6. TEST SYSTEM

6.1. Description of the cell line

Human hepatocellular carcinoma (HepG2) cell line of epithelial origin was used for the examination. The code number of the used cell line is ATCC-HB-8065, Lot N: 58210525, place of origin: Manassas, VA 20110-2209 USA. The HepG2 is a permanent cell line. It was isolated from the hepatocellular carcinoma of a 15 year old boy. This cell line has the following characteristics: high level of morphologically differentiated state, non-tumorigenic, its chromosome number is 55. HepG2 cells secret plasma proteins such as albumin, transferrin, fibrinogen and plasminogen. HepG2 cells are propagated in MEM culture liquid modified by Dulbecco, which is supplemented before use by foetal beef serum with a final concentration of 10%, and also by antibiotics with penicillin final concentration of 10 U/ ml, and streptomycin final concentration of 10 µg/ml. The cell strain culture is stored in liquid nitrogen, an ampoule cell is taken from this store and before testing it is kept in continuous culture that is used until 15 passage numbers, then a new ampoule cell is taken. The cell with batch number 58210525/5 is used for the examination.

6.2. Grounds for selecting the test system

The citotoxicity examination may be carried out using both primary and permanent cell lines. However, we endeavoured to achieve examination conditions that are the most similar to use conditions. Therefore HepG2 cell line of human origin was chosen as the examination material is also for human use.

6.3. Checking the cell line

The used HepG2 cell line is checked once a year according to the following:

- optical density values of untreated control cells are measured

- the level of how free the cell is from mycoplasma was checked, the result was negative

7. METHOD

7.1. Citotoxicity examination

7.1.1. Brief description of the method

Live, metabolically active cells absorb 3-(4-5dimethylthiazol-2yl)-2,5-diphenyltetrazolium-bromide (MTT) paint, which is then reduced to colourful formazane salts by mytocondrial dehydrogenase enzymes. The quantity of the transformed colourful formazane salt is proportional to the number of live cells, and it is soluted from the cells by izopropanol, and

measured by colorimetric method. In the citotoxicity examination, 3000 cells were located per each hole of the 96-hole cell culture pot then following 24-hour incubation a 24-hour treatment was carried out. The citotoxicity examination was carried out with undiluted kaqun water.

7.2. Preparation of examination samples

7.2.1. DMEM culture liquid prepared with Kaqun water

1.9989 g was measured from the DMEM (Dulbecco's medium w/pyruvate; GIBCO Invitrogen Corporation, Lot N:757533) powder, then soluted in 200 ml of Kaqun water, and 0.7410 g sodium-hydrogen-carbonate was added (Sigma Aldrich Kft., Lot N:BCBB8363) and mixed until dissolved, then strained through a 0.22µm Millex filter to obtain a sterile state. 20 ml inactivated foetal beef serum (10 vol%; Gibco Invitrogen Corporation; Lot N:41Q8095F) and 200 µl penicillin/streptomycin solution (10 000 U/ml penicillin and 10 000 µg/ml

streptomycin; PAA Laboratories GmbH; LotN: P01009-1954) was added to the sterile culture liquid prepared this way.

Undiluted kaqun water and this method of preparing the examination material were chosen so that we can provide the optimal quantity of nutrient needed for the growth of the cells and can investigate the 100% concentration of the examination material at the same time. In addition to this we chose this highest value as it is used in practice in this form as well.

7.2.2. DMEM culture liquid prepared with ultra-pure water

1.9975 g was measured from the DMEM (Dulbecco's medium w/pyruvate; GIBCO Invitrogen Corporation, Lot N:757533) powder, then soluted in 200 ml ultra-pure water, and 0.7405 g sodium-hydrogen-carbonate was added (Sigma Aldrich Kft., Lot N:BCBB8363) and mixed until dissolved, then strained through a 0.22µm Millex filter to obtain a sterile state. 20 ml inactivated foetal beef serum (10 vol%; Gibco Invitrogen Corporation; Lot N:41Q8095F) and 200 µl penicillin/streptomycin solution (10 000 U/ml penicillin and 10 000 µg/ml streptomycin; PAA Laboratories GmbH; LotN: P01009-1954) was added to the sterile culture liquid prepared this way.

7.2.3. Preparing positive control solution

2.5 ml DMSO (Sigma-Aldrich Kft.; Lot No: BCBB0540 was added to 50 ml DMEM (LG) W/NA PYR. (Gibco Invitrogen Corporation; Lot No: 712334) culture liquid.

7.3. Placing the examination samples in 96-hole tissue culturing pot

Column number	Description of the sample
1	Positive control (5% DMSO)
2	
3	DMEM culture liquid prepared with Kaqun water
4	
5	
6	
7	DMEM culture liquid prepared with ultra-pure water
8	
9	DMEM culture liquid (Manufacturing number: 712334)
10	Untreated control
11	Cell-free control
12	

8. Measuring the citotoxicity examination

Optical density was measured by Multiskan FC photometer (570nm/620nm). Optical density values were evaluated by Multiskan FC 2.5.1. program, and average and standard deviation were calculated by concentrations.

9. Evaluating the citotoxicity examination

9.1. Negative result

Negative result means that in the given experimental conditions the examination material does not significantly reduce the rate of viable cells compared to the untreated control.

9.2. Positive result

The examination material is citotoxic if it reduces the percentage rate of viable cells significantly in a dose-dependant way, reproducibly, and at one or more concentration levels compared to the untreated control.

10. Statistical evaluation

The data were evaluated by the Dunnett test in the one-way ANOVA statistical program running in the Graphpad computer program. The untreated control group was compared to the treated group averages.

11. Results of the citotoxicity examination

11.1. Summary table of optical density values measured at 570nm/620 nm of Plates 1 and 2

DMEM control Columns A09-H09;A10-H10		DMEM with Kaqun water Columns A03-04-05-06;H03-04-05-06				DMEM with ultra-pure water Columns A07-H07;A08-H08		Positive control A01-H01;A02-H02	
0.147	0.167	0.135	0.159	0.150	0.139	0.163	0.158	0.044	0.048
0.184	0.171	0.204	0.181	0.162	0.194	0.195	0.180	0.052	0.044
0.173	0.196	0.206	0.216	0.186	0.204	0.226	0.178	0.057	0.045
0.228	0.185	0.222	0.216	0.212	0.212	0.196	0.203	0.052	0.050
0.226	0.239	0.213	0.237	0.200	0.178	0.190	0.213	0.046	0.046
0.192	0.199	0.210	0.291	0.221	0.220	0.215	0.239	0.044	0.039
0.201	0.232	0.202	0.205	0.237	0.202	0.233	0.205	0.059	0.045
0.202	0.193	0.169	0.194	0.165	0.147	0.156	0.162	0.062	0.050
0.108	0.141	0.144	0.202	0.217	0.152	0.204	0.155	0.051	0.050
0.132	0.124	0.174	0.223	0.210	0.195	0.186	0.156	0.051	0.038
0.187	0.195	0.189	0.225	0.206	0.209	0.195	0.194	0.056	0.043
0.202	0.167	0.188	0.199	0.219	0.177	0.161	0.181	0.051	0.043
0.143	0.136	0.186	0.171	0.180	0.161	0.178	0.196	0.048	0.039
0.167	0.136	0.181	0.183	0.168	0.202	0.172	0.194	0.053	0.045
0.195	0.152	0.154	0.188	0.195	0.172	0.196	0.164	0.043	0.036
0.103	0.109	0.134	0.141	0.128	0.152	0.142	0.140	0.044	0.036
Average:0.173		Average:0.189				Average:0.185		Average:0.047	
Std		Std deviation:0.024				Std		Std	

deviation:0.031		deviation:0.021	045deviation:0.005

11.2. Summarizing evaluation of citotoxicity examination

	Measurement results		Paint reduction %	Evaluation
	Average	Std deviation		
Positive control	0.047	0.005	27.16	Positive
DMEM with Kaqun water	0.189	0.024	109.2	Negative
DMEM with ultra-pure water	0.185	0.0.21	106.9	Negative
DMEM control	0.173	0.031	100	Negative

12. Summarizing the results

In the given experimental conditions Kaqun water did not reduce the rate of viable cells compared to the untreated control.

Kaqun water does not have any citotoxic effect.

13. ARCHIVING

Examination specific documentation (Examination Plan, raw data) and non-examination specific documentation will be retained for 15 years, whereas examination material will be retained for expiry time plus 1 year. The Closing Report will not be scrapped. Archiving will take place at Molecular and Cell Biological Department of the National Institute of Chemical Safety (Budapest, Gyáli út 2-6. Building C, Groundfloor). After the given time, before destroying all materials shall be offered to the Sponsor for retaining.

Budapest, 20/12/2010

(illegible signature)

Dr. Zsuzsanna Kocsis

Annex 1

Citotoxicity examination of Kaqun water in HepG2 cells

Micro plate 1

Optical density values (570nm/620nm)

Columns 01-02: Positive control (5% DMSO)

Columns 03-06: DMEM prepared with Kaqun water

Columns 07-08: DMEM prepared with ultra-pure water

Columns 09-10: DMEM culture liquid

Columns 11-12: blind, technical control (cell-free sample)

From A to H: data of the individual parallel samples

	01	02	03	04	05	06	07	08	09	10	11	12
A	0.044	0.048	0.135	0.159	0.150	0.139	0.163	0.158	0.147	0.167	0.035	0.033
B	0.052	0.044	0.204	0.181	0.162	0.194	0.195	0.180	0.184	0.171	0.042	0.033
C	0.057	0.045	0.206	0.216	0.186	0.204	0.226	0.178	0.173	0.196	0.038	0.030
D	0.052	0.050	0.222	0.216	0.212	0.212	0.196	0.203	0.228	0.185	0.024	0.031
E	0.046	0.046	0.213	0.237	0.200	0.178	0.190	0.213	0.226	0.239	0.033	0.027
F	0.044	0.039	0.210	0.291	0.221	0.220	0.215	0.239	0.192	0.199	0.029	0.026
G	0.059	0.045	0.202	0.205	0.237	0.202	0.233	0.205	0.201	0.232	0.023	0.022
H	0.062	0.050	0.169	0.194	0.165	0.147	0.156	0.162	0.202	0.193	0.022	0.025

Concentration	Column	Description of sample	Standard deviation	Average	CV%
Positive control 5% DMSO	A01	5% DMSO	0.006	0.049	12.67
Positive control 5% DMSO	A02	5% DMSO			
DMEM with Kaqun water	A03	Kaqun	0.033	0.196	16.56
DMEM with Kaqun	A04	Kaqun			

water					
DMEM with Kaqun water	A05	Kaqun			
DMEM with Kaqun water	A06	Kaqun			
DMEM with ultra-pure water	A07	Ultra-pure water	0.027	0.195	13.74
DMEM with ultra-pure water	A08	Ultra-pure water			
DMEM culture liquid	A09	DMEM	0.026	0.196	13.02
DMEM culture liquid	A10	DMEM			
Cell-free control	A11	Cell-free control	0.008	0.031	24.26
Cell-free control	A12	Cell-free control	0.004	0.028	14.68

Number of examination: 02-CTOX-10

Annex 2

Citotoxicity examination of Kaqun water in HepG2 cells

Micro plate 2

Optical density values (570nm/620nm)

Columns 01-02: Positive control (5% DMSO)

Columns 03-06: DMEM prepared with Kaqun water

Columns 07-08: DMEM prepared with ultra-pure water

Columns 09-10: DMEM culture liquid

Columns 11-12: cell-free sample

From A to H: data of the individual parallel samples

	01	02	03	04	05	06	07	08	09	10	11	12
A	0.051	0.050	0.144	0.202	0.217	0.152	0.204	0.155	0.108	0.141	0.033	0.039
B	0.051	0.038	0.174	0.223	0.210	0.195	0.186	0.156	0.132	0.124	0.031	0.024
C	0.056	0.043	0.189	0.225	0.206	0.209	0.195	0.194	0.187	0.195	0.023	0.018
D	0.051	0.043	0.188	0.199	0.219	0.177	0.161	0.181	0.202	0.167	0.020	0.018
E	0.048	0.039	0.186	0.171	0.180	0.161	0.178	0.196	0.143	0.136	0.019	0.019
F	0.053	0.045	0.181	0.183	0.168	0.202	0.172	0.194	0.167	0.136	0.028	0.020
G	0.043	0.036	0.154	0.188	0.195	0.172	0.196	0.164	0.195	0.152	0.025	0.017
H	0.044	0.036	0.134	0.141	0.128	0.152	0.142	0.140	0.103	0.109	0.020	0.018

Concentration	Column	Description of sample	Standard deviation	Average	CV%
Positive control 5% DMSO	A01	5% DMSO	0.006	0.045	13.65
Positive control 5% DMSO	A02	5% DMSO			
DMEM with Kaqun water	A03	Kaqun	0.026	0.182	14.55
DMEM with Kaqun water	A04	Kaqun			
DMEM with Kaqun water	A05	Kaqun			
DMEM with Kaqun water	A06	Kaqun			
DMEM with ultra-pure water	A07	Ultra-pure water	0.021	0.176	11.75
DMEM with ultra-pure water	A08	Ultra-pure water			
DMEM culture liquid	A09	DMEM	0.032	0.150	21.67
DMEM culture liquid	A10	DMEM			
Cell-free control	A11	Cell-free control	0.005	0.025	21.27
Cell-free control	A12	Cell-free control	0.007	0.021	33.88

2010 - Report on the examination of KAQUN oxygen-rich water's role in reactive oxygen species generation in in vitro system /HAS/

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Report on the examination of KAQUN oxygen-rich water's role in reactive oxygen species generation in in vitro system

KAQUN Hungária Kft. and the Department of Surface Chemistry and Catalysis HAS Isotope Research Institute concluded a research contract for the examination of KAQUN oxygen-rich water in order to assess whether the clinically tested beneficial effect of the water stated for the immune system that could be supported by assessing the immunological parameters of volunteers may be evidenced at any basic level of the mechanisms or not. For this purpose we examined KAQUN oxygen-rich water's effect exercised on peroxide production and reactive oxygen species generation in a purposefully selected in vitro system, which may be important in vivo for influencing apoptotic systems. We also examined how the different effects exercised on water – heat effect, nitrogen and carbon dioxide rinse – influence KAQUN water's effect on peroxide production.

The performed examinations, their results as well as the conclusions deducted from them will be presented below, and also a recommendation for further reasonable examinations will be given.

The applied examination method

Horse radish peroxidase – peroxide – benzidine system

The principle of the method

Horse radish peroxidase produces reactive oxygen species from peroxide. This converts benzidine into a colourful product with kinoid structure. The change of colour concentration may be photometrically measured at 620 nm. Thus the system – being a highly sensitive procedure - is suitable for detecting reactive oxygen species. By the use of this method it can be assessed whether oxygen-rich water increases the quantity of reactive oxygen species in the system or not. Peroxidase first reduces molecular oxygen solved in the oxygen-rich water to peroxide, then thus produces a greater quantity of reactive oxygen species from the increased peroxide in the system.

This method may also be used for measuring antioxidant capacity. The presence of an antioxidant, e.g. ascorbic acid, poliphenol, or uric acid inhibits the formation of the colour.

Description of the process

1) Peroxidase + H_2O_2 + benzidine → reactive oxygen species + kinoidic benzidine

2) O_2 + peroxidase → H_2O_2 + benzidine → reactive oxygen species + kinoidic benzidine

Reagents

1) Horse radish peroxidase 9000 U/l

Benzidine HCl 233 µmol/l

NaCl 155 mmol/l

2) Carbamide peroxide, stabilized 2.5 mmol/l

For the examinations we prepared liophilized reagent 1) which was solved in 10 cm3 oxygen-free or –poor, ion-exchanged water. The solution is stable at a temperature between + 2 and + 8 °C for 2 weeks, and between + 15 and + 25 °C for 2 days. Reagent 2) is stabilized carbamide peroxide which was solved in 100 cm3 oxygen-free or –poor water. The solution is stable at a temperature between + 2 and + 8 °C for 1 week.

Measured samples: 200 µl KAQUN water

200 µl KAQUN water boiled for 10 minutes

200 µl KAQUN water rinsed with nitrogen

200 µl KAQUN water rinsed with carbon dioxide

200 µl oxygen-free, or –poor, ion-exchanged water control

Measuring equipment: LKB UV-Vis spectrophotometer

1 cm3 narrow cuvette

Temperature: 25 °C

We carried out the measurements in the method that 1 cm3 of reagent 1) was added to 200 µl sample, the sample was homogenized, then the reaction was started by adding 200 µl of reagent 2). Absorbance and its change were immediately measured for 3 minutes. Absorbance intensity was proportional to the quantity of the produced reactive oxygen species. Intensity measured in control water was considered 100%, the intensity measured in KAQUN water samples was compared to this.

Results and their evaluation

Examination results are represented by the attached Tables 1 and 2, and Figures 1 and 2. Based on the results it can be clearly concluded that in the applied in vitro system, in KAQUN oxygen-rich water a reactive species concentration showing the maximum may be reached in 10 seconds, whereas in the control water this process is slow, showing significantly lower maximum. The produced reactive oxygen has short life. The increase measured in comparison to the control is resulted by the fact that the oxygen-rich water allows an increase in peroxide quantity according to the reaction outlined above. We also examined whether KAQUN water's effect increasing peroxide quantity changes or not in open bottle, or to the effect of rinsing with nitrogen, carbon dioxide or boiling. From the data in Table 2 the following decreases can be assessed: 6.4% in a bottle open for 5 days, 4.7% for rinsing with nitrogen, 6.6% for rinsing with carbon dioxide, and 49.9% for boiling for 10 minutes. Boiling caused the greatest decrease of efficiency, which is naturally no surprise as the oxygen content of water increases at cooling, and decreases at heating. Whereas at a temperature of 0 °C maximum 14.5 mg oxygen can be solved in 1dm3 water, at 25 °C only 8.5 mg. KAQUN water contains 18-20 mg oxygen per dm3, which is 6 to 8 times higher than average oxygen content.

The reaction applied in in vitro system also happens the same way in the cell system as both peroxide generation from molecular oxygen and substrate oxidation take place in the cell wall while reactive oxygen is produced. Here NADH also participates in the reaction. In perfect systems there is a balance in these processes. The lack of reactive oxygen species means a problem similar to their permanent overproduction causing oxidative stress state. The extremely quick reactive oxygen increase measured in in vitro system allows the hypothesis that adding the appropriate quantity of oxygen-rich water in in vitro conditions might lead to a quick production of greater quantity of OH species in the Fenton (Haber-Weiss) reaction. It is known that several publications deal with the topic that the intracellular oxidative state, reactive oxygen species (ROS) might play an important role in apoptosis.

Programmed cell death is of high importance in the development of multi-cell living organisms and in the operation of the immune system. A great part of physiological cell death takes place by means of apoptosis, and it is a basic part of the differentiation of both animal and plant tissues. During experiments it became clear that in the development of high order organisms cell death leads to the formation of different organs, organ systems

and parts of the body, besides it plays a role in eliminating different structures used in certain development phases that are not needed any longer. Apoptosis is indisputably important in the formation of the immune system. The development of T and B lymphocytes is a complex process. During the generation of the ever renewing lymphocyte stock there will always be clones that are unable to work or are autoagressive. These need to be removed from the operating lymphocytes. This removal is of high importance so that they can function efficiently. Clones will be destroyed by means of the apoptosis mechanism. By this method the organism prevents autoimmune reactions acting against own cells. The disorders occurring in the control of the apoptotic system may lead to the generation of several diseases, mentioning just a few: autoimmune diseases, immune deficiency syndrome, rheumathoid arthritis, etc. The normal function of apoptosis is essential for wound healing as well.

Apoptosis is started and controlled by cell signals. The discussion of the complicated apoptotic cascade may not be the subject of the present report. As reactive oxygen species play an important role in this mechanism, in the sense of the above outlined processes KAQUN oxygen-rich water – if it can provide a higher concentration of molecular oxygen at cell level – may have an effect on the starting of the non-operating apoptosis, or on the increasing of the reduced apoptosis. All this can have a beneficial effect on certain diseases. It is evidenced that for example increased apoptosis have a favourable effect on rheumathoid arthritis. The clinical effects evidenced so far by KAQUN water can probably be explained by the stimulation exercised on the apoptotic process as well. The results of the examinations performed in different cell lines by using the water can be interpreted similarly.

Recommendations for further examinations

The experiment results presented in our report prove that KAQUN oxygen-rich water is able to increase the quantity of peroxide and reactive oxygen species in in vitro peroxidase – peroxide system. These experiments should be repeated in cell lines where apoptotic cascade only works weakly or it does not work at all. This means first of all the grade of apoptosis must be measured in the cell line. Several cell populations are suitable for these examinations. It is obvious to use tumour cell lines as in these the catalase linked to the membrane protects

the tumour cells from apoptosis induced by intracellular ROS. This takes place in a way that catalase decomposes peroxide very efficiently thus preventing the Fenton (Haber-Weiss) reaction in which the OH species giving apoptotic signal are generated. In the lack of peroxide, HOCl synthesis will also be inhibited, which is also an apoptotic signal, which means the tumour cell is in catalase protection. The inhibition of catalase leads to the intracellular signalisation of ROS. Thus the quantity of the produced peroxide is accumulated and Fenton (Haber-Weiss) reaction and the generation of HOCl take place, which produces reactive oxygen species.

The examination of KAQUN oxygen-rich water in cell system will probably result the fact that the quantity of the peroxide produced in the method as presented in this report – which may be reached by dosing GOX (glucose oxydase), HRP (horse radish peroxidase), and MPO (mieloperoxidase) – exceeds the effect of catalase and starts the apoptotic cascade through

the Fenton (Haber-Weiss) and HOCl. This can be evidenced by measuring the extent of apoptosis.

In in vivo conditions the question is what molecular oxygen concentration may be provided constantly from the oxygen-rich water at cell level.

Literature

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2011 - Study on the effect of Kaqun water on antioxidant capacity

NATIONAL INSTITUTE OF CHEMICAL SAFETY

DIVISION OF CHEMICAL SAFETY RESEARCH

DEPARTMENT OF MOLECULAR AND CELL BIOLOGY

Final report

Study number: 01-EXP-10

Study on the effect of Kaqun water on antioxidant capacity

2011

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Director General of NICS	Dr. Jenő Major PhD biologist	2011. 06. 21.
Study advisor	Dr. Gyula Sebestyén associate professor	2011. 06. 21.

1.GENERAL INFORMATION

1. 1. Title of the study:

Study on the effect of Kaqun water on antioxidant capacity

1.2. Introduction

Kaqun is a special water with high oxygen content, suitable for use in the form of drinking water or bathing. Kaqun water contains a high amount of oxygen in a stable, dissolved form, which can be absorbed through the skin and the digestive system, reducing hypoxia and acidosis in tissues and cells. Depending on the health status of the individual, the body can absorb different amounts of oxygen from the dissolved oxygen. In the present study we examined the effect of a regimen of bathing and drinking Kaqun water on the antioxidant parameters of healthy volunteers at the Department of Molecular and Cell Biology of the National Institute of Chemical Safety. In previous studies we have examined total antioxidant capacity in hundreds of human sera.

1.3. Aim of the study

Our aim was to study the effect of a regimen of bathing and drinking Kaqun water on the antioxidant capacity of healthy volunteers, to establish whether the treatment changes the antioxidant parameter compared to the value before treatment, and whether the gender of the subject affects the measured parameters. The studied parameters were analysed at individual and group level. The total antioxidant capacity of serum and erythrocyte lysate obtained from whole blood was evaluated, compared to the 0 point, initial values.

1.4. Study

The study was a non-GLP study, but was done according to GLP standards laid in the 9/2001. (III. 30.) joint Decree of the Ministry of Health and Ministry of Agriculture on the Application and Compliance Monitoring of Good Laboratory Practice and the OECD Principles on Good Laboratory Practice ENV/MC/CHEM (98) 17. The chemiluminescent measurement of total antioxidant capacity was done using the reagent of Diachem Ltd (Cat. No.: 48561, Office of Health Authorisation and Administrative Procedures Reg No.: HU/CA01/1678/06). The Standard Operating Procedures on the measurement of antioxidant capacity can be found at the Department of Molecular and Cell Biology.

1.5. Location of study

National Institute of Chemical Safety

Department of Molecular and Cell Biology

In vitro laboratory

1097. Budapest, Gyáli út 2-6.

TEST AND CONTROL ITEMS

2.1. Duration of the study and the procedure:

The examined persons participated in a 21 day bathing and water drinking treatment. The participants bathed once a day in individual bathtubs filled with 37 ^{o}C water containing stable oxygen, for a maximum of 50 minutes per occasion. The water drinking cure consisted of drinking 1.5 liter Kaqun drinking water every day in parallel with the baths. The bathing cure followed the standards established in the Kaqun Health Program Service. The participants had a condition assessment prior to the treatment, which was done using the Kaqun program's *Prior Condition Assessment Questionnaire* and other medical documents (e.g. previous medical reports). The prior condition assessment clarified whether there are any conditions present by which the subject is not eligible to take part in the study, such as a notifiable acute infection, e.g. active hepatitis, dysentery, salmonellosis, meningitis epidemica, anthrax, low hemoglobin level (absolute exclusion criteria) or banal acute infection (relative exclusion criteria). The participants were also questioned about regularly taken medicines.

Just before the start of the treatment an *Individual Assessment* was made, which was updated every week, as well as a *bath log*, recording the important parameters of every bathing. An individual documentation was made for every participant, denoted with a unique identifier, and stored in one place. The unique identifier serves as identification for

the following: the person himself/herself, the Kaqun institution at which the service was provided, the documentation itself, the period which the documentation applies to, and the service provided (treatment, occasional, unique). All data and information was handled strictly confidentially, treated as personal and medical data, and the documents and electronic versions of these documents were provided with adequate protection. To this end, the staff signed a confidentiality statement. The participants have the right to full information about the Kaqun treatment and they signed a *Declaration of Agreement* confirming that they had received information about the study. In the present study a special *Declaration of Agreement* (data for scientific purposes) was also signed by the participants, declaring that they had been informed about the purpose and the course of the study, and that their participation was voluntary.

The selection of 30 healthy volunteers (15 women, 15 men) was carried out.

3. METHODS:

3.1. Blood sampling:

Blood sampling at the site: day 1 before the bath, (0-point, initial value), then on days 8, 15, and 21 after the bath during the same part of the day. Blood was drawn into EDTA K2 (EDTA as coagulant) blood sampling tubes. The erythrocytes, leukocytes and platelets in blood samples anticoagulated with EDTA remain stable for 24 hours, so thus the samples are suitable for molecular diagnostic studies. The blood samples were given a unique identifier, which was marked on the sampling tube.

3.2. Specimen

3.2.1. Serum samples

For the antioxidant studies blood collection was done in vacutainer tubes with EDTA as coagulant. The tubes were marked with a unique identifier.

The whole blood samples were processed immediately after blood collection. Whole blood was centrifuged at 2500 rpm for 10 minutes. The cell-free supernatant (serum) was collected, distributed into 500 µl aliquots, and stored at -80 ^{o}C until measurement. The aliquots were marked with unique identifiers, and stored undiluted, to enable repeated measurements.

3.2.2. Erythrocyte lysates

After the removal of serum the remaining erythrocyte mass was washed with ice-cold isotonic saline 3 times to remove platelets and leukocytes, centrifuging 3 times with 2500 rpm. The pure erythrocyte mass was hemolysed with 1.5x amount of ultra-pure water. The

erythrocyte hemolysates were stored in aliquots at -80 ^{o}C until measurement of antioxidant capacity.

3.2.3. Control

1 mM ascorbic acid (Sigma-Aldrich Kft; CAS No: [50-81-7]) was used as positive control, and ultra-pure water was used as negative contol.

4. EXECUTION AND PRINCIPLE OF THE MEASUREMENT

The measurement was done according to the Standard Operating Procedures of the Department of Molecular and Cell Biology. Briefly, the measurement of antioxidant capacity (free radical binding capacity) in serum was as follows:

20 µl serum or erythrocyte lysate was pipetted into the wells of a 96 well plate, except the row for the untreated control containing 20-20 µl ultrapure water. The chemiluminescent reagent was added and mixed automatically by the instrument according to the selected protocol. Luminescence intensity is measured in each well by

summarizing the counts from 30 points. The higher the scavenger (free radical binding) capacity of the biological sample, the lower the luminescence given off by the system. Thus, the highest luminescence can be measured with ultrapure water. 4 parallels are measured of each sample.

The chemiluminescent measurement of total antioxidant capacity was done using the reagent of Diachem Ltd.

4.1. The principle of the measurement:

Briefly: In the H_2O_2/·OH microperoxidase system iron complexes cause OH• radical formation from H_2O_2 and the radical excites luminol. If a biological sample is added to the system the excitation of luminol is inhibited. There is a connection between the rate of inhibition and the redox status of the examined biological material.

The measurement was done on a Victor^3 multilabel reader (PerkinElmer). Wallac 1420 software was used to register the measured data and the parameters of the total protocol.

5. RESULTS

5.1. Evaluation of the results of serum samples

The measurement of total antioxidant capacity was done using the reagent of Diachem Ltd (Cat No: 48561). Measurement of total luminol value was done by substituting the sample with ultrapure water (100%). In the case of serum samples we measured total luminol value, using 4 parallels, and relative luminescence unit % (RLU%) was compared to 1mM ascorbate solution (Sigma-Aldrich). The Total Antioxidant Capacity (TAC) of serum samples was calculated according to the following formula: TAC %= 100-RLU% and the data for each measurement are given in appendix 1 (Table1).

5.1. Summary of the total antioxidant capacity in sera

Total Antioxidant Capacity %			
	Women	Men	Total
Increase from the start (↑↑↑)	9 60.0%	9 64.29%	18/29 62.07%
Increase from week 2 (–↑↑)	2 13.33%	1 7.14%	3/29 10.34%
Increase from week 1. then decreased (↑ – –)	4 26.66%	4 28.57%	8/29 27.58%
Total	15	14	29

5.2. Summary of the total antioxidant capacity in erythrocyte lysates

Total Antioxidant Capacity %			
	Women	Men	Total
Increase from the start (↑↑↑)	4 26.66%	3 21.43%	7/29 24.14%
Increase from week 2 (–↑↑)	3 20.0%	-	3/29 10.34%
Increase from week 1. then decreased (↑– –)	6 40.0%	-	6/29 20.69%
Increase in week 1. and 2. then decreased (↑↑–)	-	6 42.86%	6/29 20.69%
Decrease from the start (↓↓↓)	-	4 28.57%	4/29 13.79%
Other	2 13.33%	1 7.14%	3/29 10.34%
Total	15	14	29

6. STATISTICAL ANALYSIS

One-way ANOVA, and Dunnett test was used for the statistical evaluation of the results, the level of significance was set at $p<0.05$. The 1st, 2nd and 3rd week samples were compared to the initial, control values of every subject. GraphPad software was used for statistical analysis.

7. SUMMARY

Kaqun is a special water with high oxygen content, suitable for use in the form of drinking water or bathing. In our study we examined the effect of 21 days of bathing and drinking on the antioxidant parameters of healthy volunteers. The end points measured were the antioxidant capacity of serum and erythrocyte lysate obtained from whole blood. Blood sampling was done on day 1 before the treatment, (0-point), then on days 8, 15, and 21. Our aim was to study whether the treatment changes the antioxidant parameters compared to the value before treatment, and whether the gender of the subject affects the measured parameters. The studied parameters were analysed at individual and group level.

7.1. Evaluation of the total antioxidant capacity (TAC) of serum samples

We measured increased total antioxidant capacity in 72% of serum samples. In 62.07% (18/29) of the serum samples the total antioxidant capacity increased significantly at all three measured time points compared to the initial value. The increase in antioxidant status was almost identical in women and men: in the case of women it was 60.0 % (9/15) in the case of men it was 64.3 % (9/14).

In 10.34 % (3/29) of the serum samples the total antioxidant capacity did not increase after the first week of treatment, however, it increased significantly after the second and third week of treatment.

In 27.58 %-ban (8/29) of the subjects' serum samples the total antioxidant capacity increased significantly after the first and second week of treatment, and then decreased to the initial, control value.

7.2. Evaluation of the total antioxidant capacity (TAC) of erythrocyte lysates

Evaluation of the total antioxidant capacity of erythrocyte lysates obtained from whole blood showed that the antioxidant status increased in three quarters of the samples.

In 34.48% of the erythrocyte lysates the total antioxidant capacity increased from the first or the second week.

In the case of women 86.66% of the erythrocyte lysate samples showed an increase, but 40% of these decreased to the initial value at the third week.

Evaluation of the antioxidant status of erythrocyte lysates in men showed an 21.43% increase, and 78.57% of the samples showed a decrease to the initial, control values. Thus, in men, total antioxidant capacity increased in less samples, and more of the samples returned to the initial, control values, than in women.

8. CONCLUSIONS

We measured increased total antioxidant capacity in 72% of the serum samples. The evaluation of erythrocyte lysates obtained from whole blood showed that the antioxidant status increased in three quarters of the samples.

Analysing the antioxidant status of serum and erythrocyte lysate samples, we found that in both cases, the antioxidant capacity after one, two and three weeks of treatment increased significantly compared to the initial values.

9. ARCHIVING

The documentation of the study is stored in the Archives of the National Institute of Chemical Safety.

Budapest, 16. 06. 2011.

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Dr. Zsuzsanna Kocsis

Study director

Table 1.										
Total antioxidant capacity in serum samples										
Women										
No.	Code	0. week		1. week		2. week		3. week		Change
1	SzI	88,94		90,66		93,35		97,30		↑↑↑
		86,11		91,53		93,78		97,29		
		88,10		90,99		92,27		97,51		
		88,03		90,64		91,82		97,43		
		87,80	***1,20***	***90,96***	***0,42***	***92,81***	***0,91***	***97,38***	***0,11***	
				**		**		**		
2	DL	89,12		91,80		93,28		97,58		↑↑↑
		88,61		90,25		93,89		97,44		
		88,97		90,31		93,44		97,46		
		87,91		91,46		93,40		97,40		
		88,65	***0,54***	***90,96***	***0,79***	***93,5***	***0,27***	***97,47***	***0,08***	
				**		**		**		
3	SzB	88,72		91,41		93,76		97,54		↑↑↑
		87,36		92,07		93,58		97,36		
		87,81		90,65		93,25		97,50		
		87,13		91,33		93,32		97,46		
		87,76	***0,70***	***91,37***	***0,58***	***93,48***	***0,24***	***97,47***	***0,08***	
				**		**		**		
5	GÁ	88,64		90,38		90,97		96,92		_ ↑↑
		89,17		90,18		91,82		97,43		
		88,69		86,70		93,07		97,36		
		86,49		91,22		93,01		97,40		
		88,25	***1,20***	***89,62***	***2,00***	***92,22***	***1,01***	***97,28***	***0,24***	

				NS		**		**	
6	MR	88,84		90,10		93,49		97,45	↑↑↑
		87,97		90,96		94,08		97,51	
		87,78		90,96		93,81		97,55	
		87,43		91,31		93,50		97,42	
		88,01	***0,60***	***90,83***	***0,52***	***93,72***	***0,28***	***97,48***	***0,06***
				**		**		**	
7	KA	88,70		88,09		92,25		97,46	↑↑↑
		87,73		91,56		94,05		97,41	
		-		91,34		93,15		97,37	
		-		90,96		93,98		97,41	
		88,22	***0,69***	***90,49***	***1,62***	***93,36***	***0,84***	***97,41***	***0,04***
				*		**		**	
8	DP	89,39		91,77		93,58		94,10	↑↑↑
		88,11		92,87		93,77		91,67	
		82,01		93,39		88,48		92,94	
		81,84		93,20		93,98		91,87	
		85,34	***3,98***	***92,81***	***0,72***	***92,45***	***2,65***	***92,65***	***1,12***
				**		**		**	
9	JÉ	87,73		87,99		93,91		94,79	↑↑↑
		87,33		91,98		93,86		90,88	
		86,56		93,18		93,85		90,17	
		86,38		91,43		94,07		89,40	
		87,00	***0,64***	***91,15***	***2,23***	***93,92***	***0,10***	***91,31***	***2,40***
				*		**		**	

Table 1. continued												
Total antioxidant capacity in serum samples												
Women												
No.	Code	0. week		1. week		2. week		3. week		Change		
17	MG	85,29		92,87		93,26		94,72		↑↑↑		
		76,62		92,07		93,85		92,93				
		85,17		92,23		93,31		93,77				
		86,18		93,31		93,41		89,38				
		83,32	***4,49***	***92,62***	***0,58***	***93,46***	***0,27***	***92,7***	***2,33***			
				**		**		**				
19	VL	84,10		93,27		97,50		90,50		↑↑_		
		86,85		93,95		97,51		79,81				
		86,89		92,81		97,48		92,68				
		86,70		92,93		97,40		91,48				
		86,14	***1,36***	***93,24***	***0,51***	***97,47***	***0,05***	***88,62***	***5,94***			
				*		**		***NS***				
23	DN	89,67		85,78		97,26		93,11		↑↑_		
		90,76		93,26		97,63		92,67				
		90,49		93,29		96,87		93,66				
		89,87		93,12		97,15		93,27				
		90,20	***0,51***	***91,36***	***3,72***	***97,23***	***0,31***	***93,18***	***0,41***			
				NS		**		***NS***				
24	VE	91,58		93,22		97,21		92,08		↑↑_		
		91,08		93,45		97,56		89,26				
		91,35		93,27		97,14		94,03				
		91,16		93,42		97,41		93,23				
		91,29	***0,22***	***93,34***	***0,11***	***97,33***	***0,19***	***92,15***	***2,09***			
				*		**		***NS***				

28	PA	88,76		94,28		97,28		90,41		↑↑–
		91,54		93,72		96,64		91,75		
		91,55		94,16		97,44		93,15		
		91,42		94,07		97,51		92,95		
		90,82	***1,37***	***94,06***	***0,24***	***97,22***	***0,40***	***92,07***	***1,26***	
				**		**		***NS***		
29	ET	91,99		94,28		95,95		98,40		–↑↑
		91,52		94,31		96,56		99,33		
		92,26		93,61		97,56		99,25		
		91,95		89,96		97,51		98,44		
		91,93	***0,31***	***93,04***	***2,08***	***96,9***	***0,78***	***98,86***	***0,50***	
				NS		**		**		
30	KP	91,10		94,05		96,13		99,27		↑↑↑
		91,62		93,42		97,29		98,46		
		91,30		93,35		95,53		99,27		
		91,25		92,52		97,45		99,27		
		91,32	***0,22***	***93,34***	***0,63***	***96,6***	***0,92***	***99,07***	***0,40***	
				**		**		**		

Table 2.						
Total antioxidant capacity in serum samples						
Men						
No.	**Code**	**0. week**	**1. week**	**2. week**	**3. week**	**Change**
4	GJ	88,60	89,11	93,92	97,30	↑↑↑
		88,26	92,16	91,39	97,57	
		88,26	89,94	93,54	97,51	

		86,40		91,17		93,49		97,50				
		87,88	***1,00***	***90,6***	***1,34***	***93,09***	***1,15***	***97,47***	***0,12***			
				**		**		**				
10	OG	88,82		93,07		93,39		93,96		↑↑↑		
		87,29		92,00		94,12		92,39				
		86,94		93,12		93,28		94,26				
		83,94		92,91		93,87		94,44				
		86,75	***2,04***	***92,78***	***0,52***	***93,67***	***0,4***	***93,76***	***0,94***			
				**		**		**				
11	FG	87,17		93,36		93,76		94,76		↑↑↑		
		86,91		93,46		93,94		92,42				
		86,62		91,83		93,50		91,31				
		86,30		93,19		93,94		94,37				
		86,75	***0,37***	***92,96***	***0,76***	***93,79***	***0,21***	***93,22***	***1,63***			
				**		**		**				
12	HJ	86,80		93,57		93,67		94,19		↑↑↑		
		86,99		93,46		94,23		94,28				
		86,93		92,98		94,47		92,62				
		84,54		93,38		93,94		94,36				
		86,32	***1,19***	***93,35***	***0,26***	***94,08***	***0,35***	***93,86***	***0,83***			
				**		**		**				
13	LT	86,09		93,62		93,96		94,3		↑↑↑		
		85,68		93,83		94,13		94,68				
		87,08		88,71		93,89		94,38				
		86,73		90,94		93,58		93,94				
		86,40	***0,63***	***91,78***	***2,43***	***93,58***	***0,23***	***94,33***	***0,30***			
				**		**		**				
14	HL	86,32		93,32		93,87		94,98		↑↑↑		

		86,36		93,31		94,16		90,52		
		86,61		92,91		93,89		94,44		
		87,20		93,30		94,15		90,93		
		86,62	***0,41***	***93,21***	***0,20***	***94,02***	***0,16***	***92,72***	***2,32***	
				**		**		**		
15	MGy	87,16		93,78		94,14		94,90		↑↑↑
		86,73		93,82		93,82		95,05		
		86,83		93,36		94,16		94,94		
		87,24		93,67		94,28		93,14		
		86,99	***0,25***	***93,66***	***0,21***	***94,1***	***0,20***	***94,51***	***0,91***	
				**		**		**		

Table 2. continued

Total antioxidant capacity in serum samples

Men

No.	Code	0. week		1. week		2. week		3. week		Change
16	KL	86,97		93,08		93,50		-		↑↑-
		82,48		92,08		93,94		-		
		82,44		93,65		93,70		-		
		86,27		93,67		93,61		-		
		84,54	***2,42***	***93,12***	***0,75***	***93,69***	***0,19***	***-***	***-***	
				**		**		-		
18	VCs	82,60		93,16		97,62		94,54		↑↑↑
		84,61		92,38		97,37		94,04		
		87,08		92,82		96,97		93,35		
		86,27		86,69		97,37		93,87		
		85,14	***1,98***	***91,26***	***3,07***	***97,33***	***0,27***	***93,95***	***0,49***	

				**		**		**		
20	VG	87,30		93,80		97,35		91,30		↑↑–
		86,93		93,35		97,14		82,86		
		87,22		93,14		97,41		93,66		
		87,30		92,65		97,51		85,55		
		87,19	***0,18***	***93,24***	***0,48***	***97,35***	***0,16***	***88,34***	***5,00***	
				*		**		***NS***		
21	PG	91,83		93,43		97,45		92,31		↑↑–
		88,23		93,23		97,53		83,24		
		91,40		87,57		97,11		93,29		
		91,60		92,83		97,28		87,76		
		90,77	***1,70***	***91,77***	***2,81***	***97,34***	***0,19***	***89,15***	***4,62***	
				NS		*		***NS***		
22	MEGy	89,11		92,63		97,48		94,29		↑↑–
		91,75		92,86		97,50		89,80		
		91,97		92,70		97,11		91,73		
		91,45		93,55		97,29		90,60		
		91,07	***1,32***	***92,94***	***0,42***	***97,35***	***0,18***	***91,61***	***1,96***	
				NS		*		***NS***		
25	PI	91,86		93,31		96,76		93,88		–↑↑
		92,38		93,28		97,32		93,86		
		92,03		93,15		93,47		93,90		
		90,39		93,32		97,49		93,67		
		91,67	***0,88***	***93,27***	***0,08***	***96,26***	***1,89***	***93,83***	***0,11***	
				NS		**		*		

26	ZsI	91,96		92,62		97,40		91,14		↑↑_
		91,64		93,49		97,47		90,31		
		89,03		89,71		97,02		90,81		
		90,14		92,73		97,02		92,27		
		90,69	***1,36***	***92,14***	***1,66***	***97,23***	***0,24***	***91,13***	***0,83***	
				NS		**		***NS***		
27	BZs	91,30		93,27		97,62		93,38		↑↑↑
		90,99		93,33		97,49		94,02		
		90,38		92,94		97,29		92,48		
		90,66		93,41		97,44		93,42		
		90,83	***0,40***	***93,24***	***0,21***	***97,46***	***0,14***	***93,33***	***0,63***	
				**		**		**		

Table 3.

Total antioxidant capacity in erythrocyte lysates

Women

No.	Code	0. week		1. week		2. week		3. week		Change
1	SzI	0,50513		0,50675		0,50766		0,53050		_↑↑
		0,50503		0,50667		0,50759		0,53039		
		0,50505		0,50537		0,50759		0,53045		
		0,50481		0,50400		0,50761		0,53046		
		0,505	***0,00014***	***0,506***	***0,0013***	***0,508***	***0,00003***	***0,5300***	***0,00005***	
				NS		**		**		
2	DL	0,50130		0,52881		0,57978		0,59211		↑↑↑

		0,50135		0,52797		0,57988		0,59202		
		0,50119		0,52875		0,57997		0,59211		
		0,50125		0,52887		-		0,59190		
		0,501	***0,00007***	***0,529***	***0,00004***	***0,580***	***0,00009***	***0,592***	***0,0001***	
				**		**		**		
3	SzB	0,49273		0,55080		0,54935		0,48255		↑↑↓
		0,49262		0,55081		0,54921		0,48260		
		0,49254		0,55017		0,54931		0,48254		
		0,49258		0,55085		0,54929		0,48254		
		0,493	***0,00008***	***0,551***	***0,00003***	***0,549***	***0,00006***	***0,483***	***0,00003***	
				**		**				
5	GÁ	0,50746		0,52393		0,43678		0,52182		↑↓↑
		0,50739		0,52242		0,43679		0,52181		
		0,50737		0,52390		0,43678		0,52176		
		0,50708		0,52379		0,43685		0,52176		
		0,5h07	***0,00017***	***0,524***	***0,00007***	***0,437***	***0,00004***	***0,522***	***0,00003***	
				**				**		
6	MR	0,50658		0,50054		0,52259		0,55696		_↑↑
		0,50658		0,50032		0,52247		0,55692		
		0,50660		0,50053		0,52242		0,55690		
		0,50627		0,50059		0,52224		0,55689		
		0,507	***0,00016***	***0,500***	***0,00001***	***0,522***	***0,00015***	***0,557***	***0,00003***	
				NS		**		**		
7	KA	0,84901		0,53324		0,54221		0,54982		↑↑↑

		0,84902		0,53291		0,54221		0,54980		
		0,84833		0,53317		0,54215		0,54975		
		0,84897		-		0,54201		0,54977		
		0,849	***0,00 034***	***0,53 3***	***0,00 02***	***0,542***	***0,0000 7***	***0,55 0***	***0,0 0003***	
8	DP	0,42211		0,46211		0,50836		0,48833		↑↑↑
		0,42210		0,46157		0,50825		0,48832		
		0,42204		0,46180		0,50821		0,48820		
		0,42211		-		0,50822		0,48794		
		0,422	***0,00 003***	***0,46 2***	***0,00 03***	***0,508***	***0,0000 7***	***0,48 8***	***0,0 0018***	
				**		**		**		
9	JÉ	0,49023		0,52473		0,53414		0,49153		↑↑↓
		0,49024		0,52390		0,53410		0,49152		
		0,49010		0,52468		0,53402		0,49155		
		0,49017		0,52439		0,53407		0,49145		
		0,49	***0,00 006***	***0,52 4***	***0,00 04***	***0,534***	***0,0000 5***	***0,49 2***	***0,0 0004***	
				**		**				

Table 3. continued													
Total antioxidant capacity in erythrocyte lysates													
Women													
No.	**Code**	**0. week**		**1. week**		**2. week**		**3. week**	**Change**	
17	MG	0,52310		0,55358		0,53707		0,51401	↑↑↓	
		0,52309		0,55360		0,53701		0,51399		
		0,52298		0,55352		0,53647		0,51393		
		0,52313		0,55279		0,53692		0,51393		
		0,523	***0,00007***	***0,553***	***0,00039***	***0,537***	***0,00027***	***0,514***	***0,00004***	
				**		**				
19	VL	0,51556		0,54731		0,53028		0,55675	↑↑↑	
		0,51556		0,54725		0,52989		0,55673		
		0,51556		0,54706		0,53029		0,55658		
		0,51559		0,54729		0,53024		0,55657		
		0,516	***0,00001***	***0,547***	***0,00011***	***0,530***	***0,00019***	***0,557***	***0,00009***	
				**		**		**		
23	DN	0,48894		0,51967		0,53343		0,47792	↑↑↓	
		0,48891		0,52047		0,53341		0,47787		
		0,48888		0,52052		0,53349		0,47785		
		0,48885		0,52020		0,53332		0,47785		
		0,489	***0,00004***	***0,562***	***0,00007***	***0,533***	***0,00007***	***0,487***	***0,00004***	
				**		**				
24	VE	0,52267		0,53108		0,54039		0,43546	↑↑↓	
		0,52260		0,53086		0,54029		0,43542		

		0,52262		0,53106		0,54032		0,43546					
		0,52257		0,53102		0,54026		0,43545					
		0,523	***0,00004***	***0,531***	***0,0001***	***0,540***	***0,00006***	***0,435***	***0,00002***				
				**		**							
28	PA	0,54578		0,53382		0,58224		0,55904		↓↑↑			
		0,54571		0,53470		0,58228		0,55939					
		0,54570		0,53475		0,58223		0,55934					
		0,54524		-		0,58214		0,55945					
		0,546	***0,00025***	***0,534***	***0,00052***	***0,582***	***0,00006***	***0,559***	***0,00004***				
						**		**					
29	ET	0,51867		0,54275		0,60786		0,51689		↑↑↓			
		0,51864		0,54250		0,60780		0,51667					
		0,51881		-		0,60771		0,51555					
		0,51806		-		0,60774		0,51528					
		0,519	***0,00033***	***0,543***	***0,00018***	***0,608***	***0,00007***	***0,516***	***0,008***				
				**		**							
30	KP	0,54435		0,53936		0,48379		0,45411		↓↓↓			
		0,54432		0,53934		0,48372		0,45419					
		0,54417		-		0,48369		-					
		-		-		0,48359		-					
		0,544	***0,0001***	***0,539***	***0,00001***	***0,484***	***0,00008***	***0,454***	***0,00006***				

Table 4.										
Total antioxidant capacity in erythrocyte lysates										
Men										
No.	**Code**	**0. week**		**1. week**		**2. week**		**3. week**		**Change**
4	GJ	0,53547		0,4250		0,54310		0,42214		↓↑↓
		0,53545		0,42425		0,54304		0,42207		
		0,53535		0,42497		0,54308		0,42208		
		0,53543		0,42457		0,54309		0,42210		
		0,535	***0,00005***	***0,425***	***0,00036***	***0,543***	***0,00003***	***0,422***	***0,00003***	
10	OG	0,44128		0,55613		0,50731		0,45982		↑↑↑
		0,44128		0,55614		0,50720		0,45978		
		0,44121		0,55575		0,50729		0,45976		
		0,44121		-		0,50727		0,45975		
		0,441	***0,00004***	***0,556***	***0,00022***	***0,507***	***0,00005***	***0,460***	***0,00003***	
				**		**		**		
11	FG	0,50800		0,53994		0,48499		0,49754		↑↓↓
		0,50773		0,54007		0,48015		0,49762		
		0,50792		-		0,48494		0,49760		
		0,50791		-		0,48505		0,49749		
		0,508	***0,00011***	***0,540***	***0,00009***	***0,485***	***0,00004***	***0,498***	***0,00006***	
				**						
12	HJ	0,49675		0,51993		0,480051		0,50694		↑↓↓
		0,49666		0,52037		0,480052		0,50699		
		0,49672		0,52028		0,480034		0,50696		
		0,49676		-		-		0,50695		

		0,497	***0,00004***	***0,520***	***0,00023***	***0,480***	***0,00001***	***0,507***	***0,00004***	
				**						
13	LT	0,53241		0,56250		0,42510		0,52529		↑↓↓
		0,53241		0,56244		0,42499		0,52530		
		0,53237		0,56254		0,42514		0,52523		
		0,53238		0,56238		0,42499		0,52520		
		0,532	***0,00002***	***0,562***	***0,00007***	***0,425***	***0,00008***	***0,525***	***0,00005***	
				**						
14	HL	0,32708		0,53251		0,51953		0,53994		↑↓↑
		0,52668		0,53249		0,51948		0,53992		
		0,52699		0,53206		0,51953		0,53992		
		0,52700		0,53250		0,51903		0,53982		
		0,527	***0,00018***	***0,532***	***0,00022***	***0,520***	***0,00003***	***0,540***	***0,00005***	
				**				**		
15	MGy	0,43996		0,53425		0,55616		0,54011		↑↑↑
		0,43991		0,53444		0,55608		0,54012		
		0,43963		0,53439		0,55608		0,54008		
		0,43961		-		0,55616		0,53997		
		0,44	***0,00018***	***0,534***	***0,0001***	***0,556***	***0,00005***	***0,540***	***0,00007***	
				**		**		**		
16	KL	0,52643		0,53355		0,51829		-		↑↓-
		0,52642		0,53356		0,51828		-		
		0,52632		0,53352		0,51823		-		
		0,52641		0,53354		0,51810		-		
		0,526	***0,00005***	***0,534***	***0,00002***	***0,518***	***0,00009***	***-***	***-***	

				**		-	

Table 4. continued

Total antioxidant capacity in erythrocyte lysates

Men

No.	Code	0. week		1. week		2. week		3. week		Change
18	VCs	0,53078		0,53372		0,55264		0,52596		↑↑↓
		0,53075		0,53370		0,55255		0,52586		
		0,53078		0,53357		0,55261		0,52590		
		0,53023		0,53369		0,55264		0,52583		
		0,531	***0,00027***	***0,534***	***0,00007***	***0,553***	***0,00004***	***0,526***	***0,00006***	
						**				
20	VG	0,54318		0,48997		0,50028		0,53122		↓↓↓
		0,54317		0,48999		0,50022		0,53119		
		0,54318		0,48995		0,50030		0,53118		
		0,54321		0,48975		0,50029		0,53116		
		0,543	***0,00002***	***0,490***	***0,00011***	***0,500***	***0,00003***	***0,531***	***0,00002***	
21	PG	0,51034		0,53631		0,44810		0,50049		↑↓↓
		0,51040		0,53623		0,44811		0,50088		
		0,51027		0,53631		0,44805		0,50088		
		-		0,53633		0,44799		0,50083		
		0,51	***0,00006***	***0,536***	***0,00004***	***0,448***	***0,00005***	***0,501***	***0,00019***	
				**						
22	MEGy	0,51565		0,42426		0,54177		0,54802		↓↑↑
		0,51587		0,42424		0,54143		0,54795		

		0,51589		0,42416		0,54182		0,54794		
		0,51580		-		0,54174		0,54794		
		0,516	***0,00011***	***0,424***	***0,00005***	***0,542***	***0,00018***	***0,548***	***0,00004***	
						**		**		
25	PI	0,55327		0,51860		0,50703		0,47039		↓↓↓
		0,55322		0,52023		0,50703		0,47039		
		0,55293		-		0,50699		0,47029		
		-		-		0,50697		0,47033		
		0,553	***0,00018***	***0,519***	***0,00115***	***0,507***	***0,00003***	***0,470***	***0,00005***	
26	ZsI	0,52689		0,46993		0,44421		0,43275		↓↓↓
		0,52690		0,47000		0,44413		0,43271		
		0,52688		0,46908		0,44416		0,43276		
		0,52670		-		0,44405		0,43235		
		0,527	***0,0001***	***0,470***	***0,00052***	***0,444***	***0,00007***	***0,433***	***0,0002***	
27	BZs	0,48398		0,53134		0,54595		0,53493		↑↑↑
		0,48391		0,53124		0,54589		0,53492		
		0,48392		0,52949		0,54590		0,53495		
		0,48392		-		0,54580		0,53491		
		0,484	***0,00003***	***0,531***	***0,00104***	***0,546***	***0,00006***	***0,535***	***0,00002***	
				**		**		**		

Abbreviations:

NS= not significant

*=p<0.05

**=p<0.01

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10. Kocsis Zsuzsanna**,** Marcsek Zoltán

Antioxidánsok az elsődleges megelőzésben

Corvinus Egyetem Budapest, Fővárosi Egészségnap, 2008. április 29.

2012 - Report about effects of Kaqun water on the speed of cognitive functions

Report about effects of Kaqun water on the speed of cognitive functions

Permit Number: IV-R-015-14-4/2012

Summary

Our research project „The effects of Kaqun water on the speed of cognitive functions" was conducted on randomly selected healthy elderly individuals who fit the age group. The average age of the groups was around 65 years. We made 4 groups, 1.5l; 1l; 0.5l daily consumption of Kaqun water as well as the control group whose members drank 1l tap water. During the measurements the people who made the measurements and who assessed the results did not know who was in which group, it was only made known to them after the data have been processed (double blind). The connection between the dose and the therapeutic effect has also been examined.

We examined: plethysmogram, the spread of the pulse relaxed and under load with tools of HRV analysis; blood pressure (systolic and diastolic), oxygen saturation, SRT (reaction time) and CRT (cognitive reflex time).

The results of the measurements and the significance of the changes were assessed by RopStat software.

We have gotten significant results for blood pressure lowering effect (systolic, diastolic).

By examining the spread of the pulse we managed to determine the stress index. In the 1.5l and 1l daily groups significance could be shown in some intervals.

In case of the decreasing of the reflex time, we have gotten significant results in all three groups.

In case of the cognitive time we have gotten significant results in all three groups.

The oxygen saturation has only increased significantly in the 1,5l group.

The data of the control group have shown similar results to the consumption of Kaqun water in the base data-first week interval in several cases, but this effect is not lasting. The cause of this might lie in the psychic area, but more likely that by ceasing the lack of fluids, the circulating blood volume is diluted that's why improvements are shown.

This improved effect is not as lasting as the effect caused by consumption of Kaqun water.

By examining the dose and effect duration it can be seen that in case of the 1-1.5l/day the maximal effects are shown in the 3rd-4th week, there is constant improvement, while in case of 0.5l/day consumption the best results are in the 2nd week, after that the results deteriorate. This signifies that basically 1-1.5l/day dose is appropriate.

We can see from this examination that Kaqun water improves the haemodynamics, it speeds up the reflex and thought processess, increases the body's oxygen content in case of elderly people.

Introduction

The theory of Kaqun water

Kaqun water is specially produced water for consumption and bathing (functional water), whose physical properties, ph, oxygen level are different from normal drinking water (OTH permit 420-2/2007, OKI expert opinion: 6212/2011). Kaqun water is a fluid, which contains 16 mg oxygen per litre, pH value is between 7.5 and 8.5 (slightly alkaline), it has lower osmotic pressure than cytoplasm, whose effect mechanism is:

- modified absorption and utilization conditions
- high dissolved oxygen content
- burst-like pro-oxidant effect, which speeds up cell regeneration, boosts the immune system, causes vasodilation and potentiates the body's antioxidant enzyme system
- alkaline effect, which decreases acidic deposition, and through this tissue edema

We associate the deceleration of memory, neural and cognitive functions with old age. We assume this is due to the accumulation of pro-oxidant radicals, metabolites accumulating in the body and the decrease of neural and mental activity.

The decline of neural and mental functions is one of the early signs of aging, which can be objectively determined by measurements.

Aim:

1. To justify or reject the hypothesis that the consumption of Kaqun water influences:
    a. basic mental functions
    b. impact on the operation of the autonomic nervous system
    c. influences blood pressure
    d. effects vasodilation
2. To examine whether these effects depend on the dosage
3. To examine the rate of development in time and durability of the effects

Materials and methods

The examination was led by András Huszár dr. PhD, implemented in practice by Iván Szalkai dr., with the involvement of the workers of Kaqun Ltd. A total of 60 people took part. They formed 4 groups with 15 people each. The groups were:

1. Consumption of 0.5l Kaqun water daily
2. Consumption of 1l Kaqun water daily
3. Consumption of 1.5l Kaqun water daily
4. Control group; consumption of 1l water daily

Table 1. Group characteristics

	composition			age	
	male	female	total	average	standard deviation
1.	5	8	13	65,69 years	4,73
2.	3	12	15	63,73 years	6,56
3.	3	8	11	68,36 years	6,12
4.	2	7	9	66.44 years	7,9
total / average	13	35	48	65,93 years	6,33

The dropout during the examination was not due to side effects. One volunteer complained about headache, but relationship with the water consumption could not be proven.

The study was a **placebo controlled, randomized, double blind trial.**

The materials: Kaqun water, placebo; tap water in Kaqun glass.

The study included volunteers of both sexes between 50 and 75 years of age, who did not consume Kaqun water nor bathed in Kaqun water for 2 months prior to the examination. Health status was appropriate for their age. The sorting of the people was done in order of arrival, no other factor determined it (0.5 – 1 – 1.5), random method. Members of the control group were chosen from visitors from another town, they did not meet with the real group.

When selecting the sample, the following criteria had to be fulfilled by the volunteers.

Self-sufficient, or still active worker in the given age group, lives an active social life, has average health status, (non-hospital treatment) elderly for this study.

The examination consisted of the following tests:

1. Serial reflex time (SRT) – testing the dominant hand 35 times. We analyzed the average P200 time, filtering out the 3 highest values we deem as a learning phase. We also examine the wave of the P200 time. Normal value is 200msec.
2. Cognitive reflex time (CRT) – recognizing different sounds, signaling with the push of a button, making it more difficult with counting backwards, pushing the button and simultaneously saying the number. Length of the test is 35 times. Normal value is 300 msec. In the examination we did not include the 3 highest values and values

under 200msec. We deemed the highest value a learning value, which falsely stretches the results and the values under 200msec are not the results of a cognitive process.

3. HVR measurement, standard deviation, standard deviation % in normal condition and after 10 squats (30 watt load). We recorded base data and the differences. The standard deviation data represents the stimuli of the sympathetic and parasympathetic nervous system, so can ve used as stress index. We determine the minimal and average value of vasodilation, which shows the flexibility of the capillaries.

4. Measuring oxygen saturation

5. Blood pressure and heart rate were recorded. The heart rate was measured in a relaxed state and after load in an every 10 second cycle, the fit index, ie. the time when the heart rate reached the relaxed heart rate after load.

Instruments for measuring:

Oxygen saturation: Innomed joint-stock company Oxycard device, which records the oxygen saturation of the peripheral blood and the average heart rate.

Other tests: Kellényi's tremometer, which records the time between a signal and the response, also a software can dynamically record the measures valued after statistical analysis.

Statistical analysis: FFT analysis, linear correlation- and regression analysis, standard error analysis, normality test, dependent variables (equality of averages test, stochastic homogeneity test), and to assess the significance level of the changes.

The duration of the test was **½** year.

Groups were formed from the subjects, and the average (median) values of the groups were analyzed.

Patient monitoring and impact assessment

5 measurements were performed on the selected subjects, at the beginning, then on the 7th, 14th, 21st, and 28th day. The fluid supply those days was dine with calculated quantities (3, 5, 7 bottles of water). The data we measured are kept both in electronic form and in a paper dossier, group composition was recorded separately. People who made the measurements were not did not know the group composition.

Among the paper documents are stored the certificate for voluntary participation in the study, general information document, examination sheet and certified receipt of the water.

Test results

1. **Evaluation of systolic blood pressure**

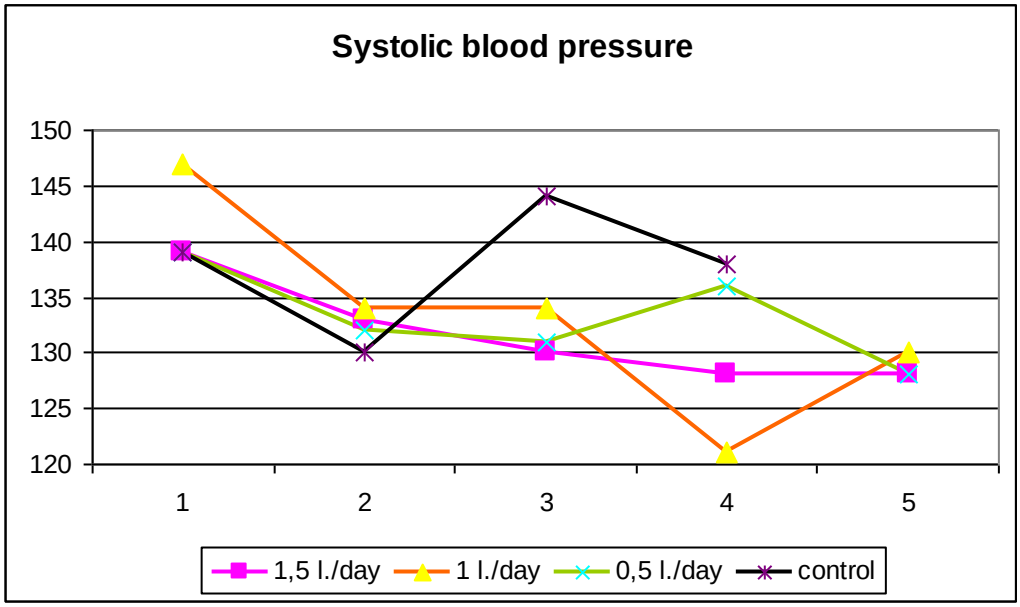
   The blood pressure was measured before everything else, after at least 10 minutes rest. The results are as follows (comparison of median values):

Table 2. Comparative data

change of systolic blood pressure median value						rate of decrease
weeks	base	1	2	3	4	
1,5 l./day	139	133	130	128	128	11
1 l./day	147	134	134	121	130	(26), 17
0,5 l./day	139	132	131	136	128	11
control	139	130	144	138		(9), 1

The biggest rate in the decrease of the systolic blood pressure occurred in the 1l group, in the control group it was minimal.

Graph 1. Systolic blood pressure



The analysis clearly shows that a significant reduction of the blood pressure can be achieved in all 3 groups, while the control group only moves with the test groups in the first week, later it goes back to the base value (psychic effect, filling of water bodies).

1,5l/day group analysis:

Table 3. 1.5l/day group data

group	average	median	standard deviation	relative deviation	normality (norm=1)
base	142	139	18,52566	0,13	0,9891
1. week	131,45	133	11,55304	0,0879	0,9778
2. week	129,45	130	10,99421	0,0849	0,9785
3. week	124	128	13,29662	0,107	0,7428
4. week	130,64	128	16,98984	0,13	0,8006

When testing the **dependent variables** the equality of averages were tested (analysis of variance, robust analysis of variance with degrees of freedom correction, Geisser-Greenhouse analysis, Huynh-Feldt analysis), significance level $p<0.001$

Stochastic homogeneity test (Friedman test, analysis of variance by ranking numbers, robust analysis of variance with degrees of freedom correction, Geissel-Greenhouse analysis, Huynh-Feldt analysis), significance level $p<0.005$

Table 4. Significance level of linear correlation

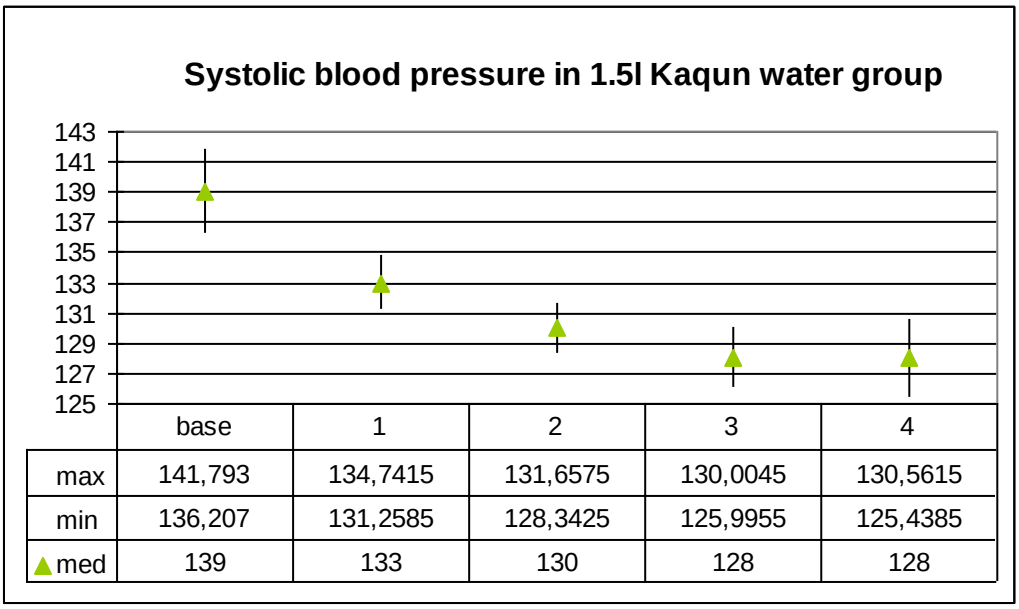
Pearson's correlation coefficient

	1	2	3	4
base	p=0,2159	p=0,2624	p=0,1550	p=0,0292
1		p=0,0385	p=0,0054	p=0,0044
2			p=0,2504	p=0,0046
3				p=0,0471
4				

(white: not significant, yellow: $p<0.05$, green: $p<0.01$, blue: $p<0.001$)

The systolic blood pressure started significantly decreasing at the end of the second week, and kept that until the end of the study.

Graph 2. 1.5l group standard error changes



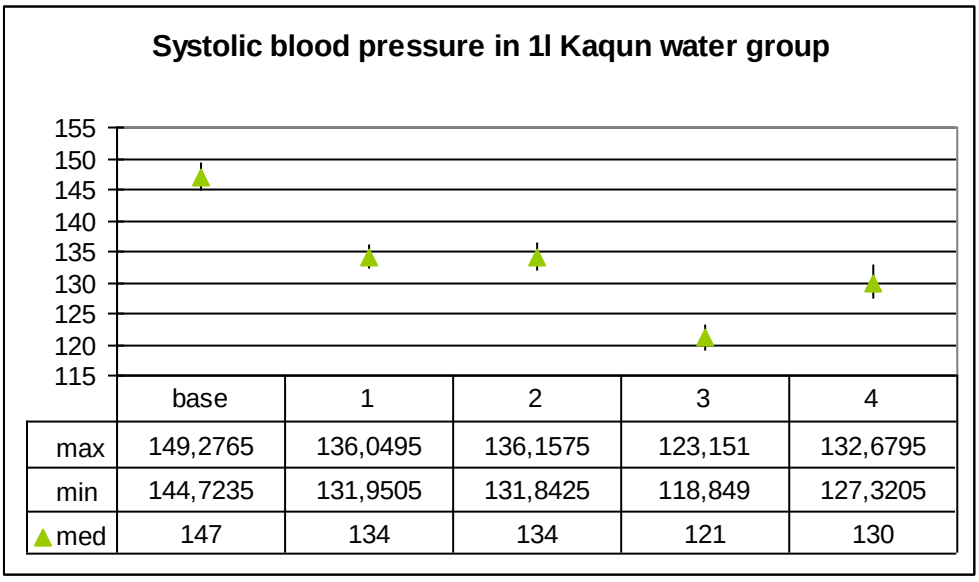
	base	1	2	3	4
max	141,793	134,7415	131,6575	130,0045	130,5615
min	136,207	131,2585	128,3425	125,9955	125,4385
▲med	139	133	130	128	128

From the second week onwards, the measured values fall outside the margin of the standard error; this indicates that there is an effect behind it, and not measurement fluctuation.

Evaluation: the dependent variables and the stochastic test proved that the grouping was correct. The linearity test examines the difference between each phase, so it determines the therapeutic period. From this we can see that the biggest change occurs related to the second week of the treatment, when the third and fourth week values are in strong significance. At the fourth week there is significant change in every comparison.

1l/day group analysis

Graph 3. 1l/group standard error analysis



	base	1	2	3	4
max	149,2765	136,0495	136,1575	123,151	132,6795
min	144,7235	131,9505	131,8425	118,849	127,3205
▲med	147	134	134	121	130

The change in the values is outside of the boundary of the standard error at the first week already. The decrease continues until the third week, when the blood pressure rises, but does not reaches the base value.

Table 5. 1 l/day data

group	average	median	standard deviation	relative deviation	normality (norm=1)
base	140,4	147	17,63438	0,126	0,721
1. week	136,8667	134	15,87391	0,116	0,9505
2. week	136,2	134	16,7127	0,123	0,9982
3. week	126,8	121	16,66133	0,131	0,6611
4. week	132,4667	130	20,75664	0,157	0,7439

The average decrease in the systolic blood pressure is 8 mmHg (best value 14 mmHg), median 17 mmHg (best value 26mmHg). At the last measurement, the blood pressure increased. The base value, third and fourth week values were not of normal distribution.

When testing the **dependent variables** the equality of averages were tested (analysis of variance $p<0.001$, robust analysis of variance with degrees of freedom correction, Geisser-Greenhouse analysis, Huynh-Feldt analysis), significance level $p<0.005$

Stochastic homogeneity test (Friedman test, analysis of variance by ranking numbers, robust analysis of variance with degrees of freedom correction, Geissel-Greenhouse analysis, Huynh-Feldt analysis), significance level $p<0.005$

Table 6. Significance level of linear correlation

Pearson's correlation coefficient

	1	2	3	4
base	p=0,0178	p=0,0009	p=0,0035	p=0,1185
1		p=0,0005	p=0,0051	p=0,0000
2			p=0,0008	p=0,0025
3				p=0,0139
4				

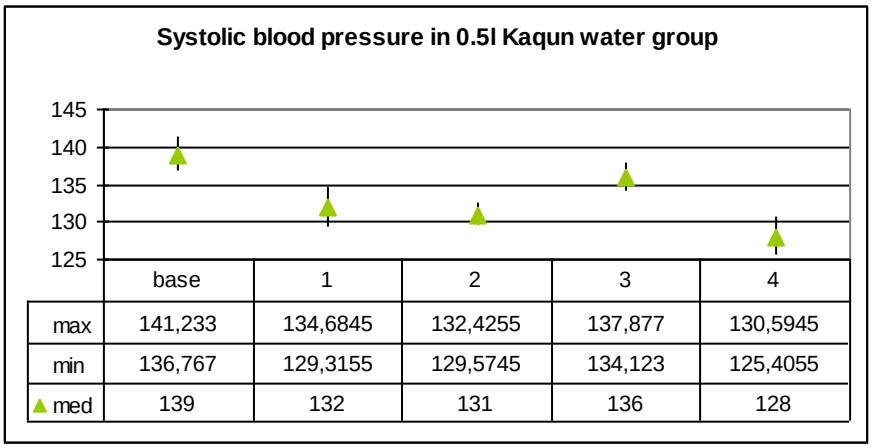
(white: not significant, yellow $p<0,05$, green: $p<0,01$, blue: $p<0,001$)

Evaluation: While the dataset is different from normal, the dependent variables and the stochastic test proved that the grouping was correct. The linearity test examines the

difference between each phase, so it determines the therapeutic period. From this we can see that the change from the second week (blue) indicates very high significance. When compared to the base value, the difference is significant except for the last week and this stands related to all previous values as well (we evaluate increase in the fourth week).

0.5l/day group analysis

Graph 4. 0.5l group standard error analysis



The change in the values is outside the boundary of the standard error from the first week already. The standard deviation is biggest in the first and fourth weeks, which signifies a slower and less lasting process of the decrease of the blood pressure.

Table 7. 0.5 l/day data

group	average	median	standard deviation	relative deviation	normality (p)
base	139	139	16,1	0,116	1
1. week	133,6923	132	19,36	0,145	0,9902
2. week	132	131	10,28	0,0779	0,7223
3. week	133,9231	136	13,54	0,101	0,4314
4. week	129,7692	128	18,71	0,144	0,646

The average decrease in the systolic blood pressure in the 0.5l group was 10 mmHg, 11mmHg in median. The biggest standard deviation is after the first week (different reactions), the deterioration in the normailty test can be seen at the third week measurement, where by on patient we measured a 28 mmHg decrease in the blood pressure. Due to this data only 3 patients were in the below average group.

The examination of the **dependent variables** and the **stochastic homogeneity** didn't show any significance even by 20% trim level.

Table 8. Significance level of linear correlation

Pearson's linear correlation coefficient

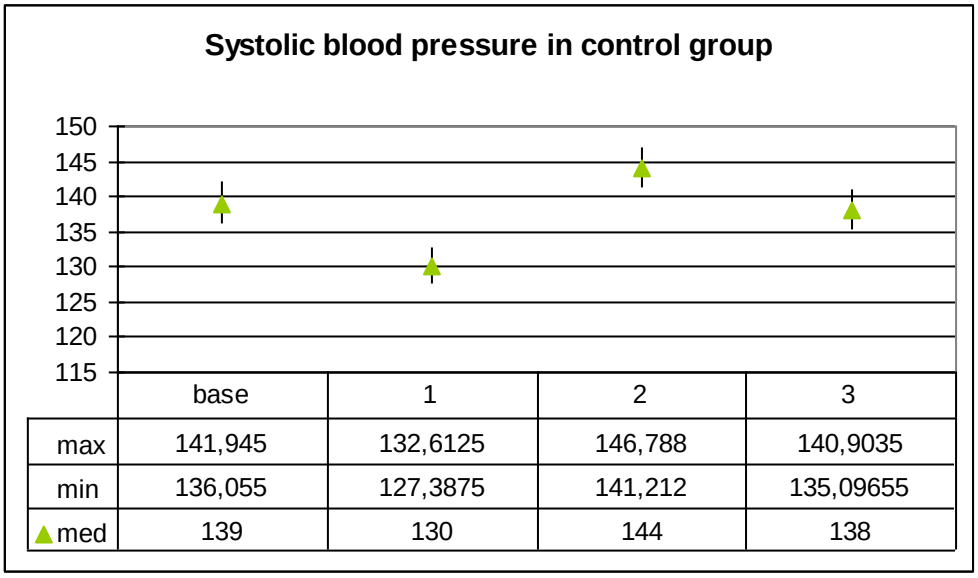
	1	2	3	4
base	p=0,0096	p=0,0330	p=0,0310	p=0,0096
1		p=0,0571	p=0,0006	p=0,0240
2			p=0,0235	p=0,0020
3				p=0,0223
4				

(white: not significant, yellow p<0,05, green: p<0,01, blue: p<0,001)

Examination of the linear correlation shows a significant change, and the significance is particularly high when comparing it to the base value.

Control examination

Graph 5. Control group systolic blood pressure standard error analysis



Significant decrease in the blood pressure can be observed, which exceeds the base value in the second week then sets back to it in the third week. The first decrease is likely due to the filling of the water bodies and the body compensates this effect and the blood pressure increases again.

Summary:

The Kaqun water significantly reduces the systolic blood pressure. For the 1.5l/day group this continuously applied during the consumption, in the 1l/day group this effect was not so lasting, a slight increase can be observed in the last week, then the decrease continues. In the 0.5l group this jump can be observed in the third week, then the decrease continues. It can be concluded that the effect is proportional to the quantity consumed.

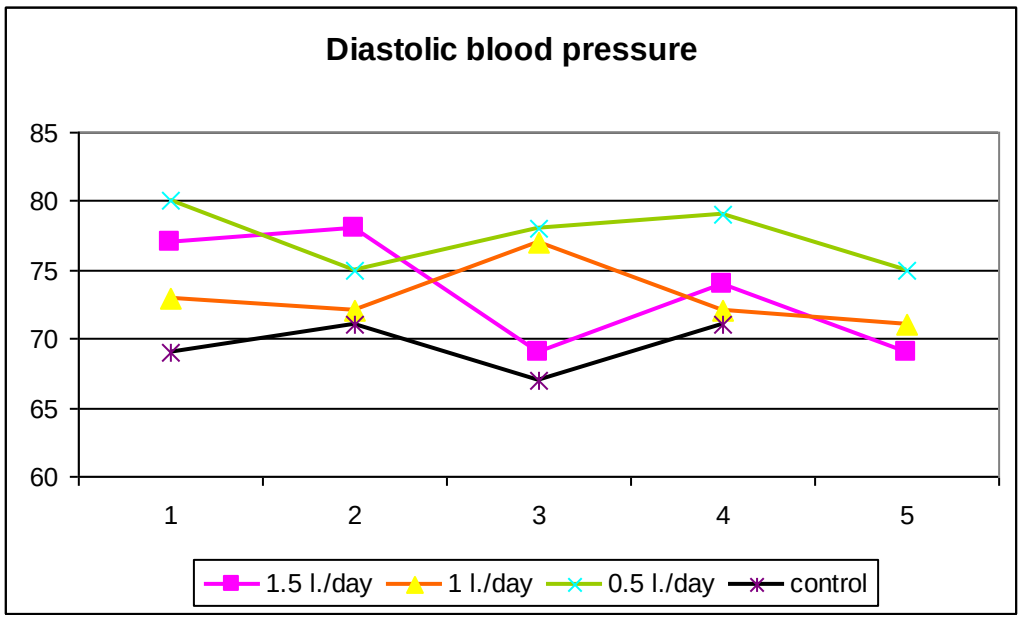
Evaluation of diastolic blood pressure

The blood pressure was measured before everything else, after at least 10 minutes rest. The results are as follows (comparison of median values):

Table 9. Diastolic values

change of diastolic blood pressure median					
weeks	**Base**	**1**	**2**	**3**	**4**
1.5 l./day	77	78	69	74	69
1 l./day	73	72	77	72	71
0.5 l./day	80	75	78	79	75
control	69	71	67	71	

Graph 6. Change of diastolic values



When measuring the diastolic values, the decreasing tendency of the blood pressure could be detected, though in an undulating manner.

1.5l group analysis

Table 10. Effect of 1.5l on diastolic blood pressure

group	average	decrease b-x	median	decrease b-x	standard deviation	relative deviation	normality (p)
base	78,90909		77		10,22	0,13	0,9901
1. week	75,81818	3,09091	78	1	9,806	0,129	0,9895
2. week	71,09091	7,81818	69	8	9,104	0,128	0,9483
3. week	76,18182	2,72089	74	3	6,539	0,0858	0,8846
4. week	72	6,90909	69	8	10,22	0,142	0,8878

Checking the median, the diastolic pressure does not change after the first week; it reaches the lowest value by the second week.

The analysis of the **dependent variables**, did not show any significance, in case of the **stochastic homogeneity test** the significance is only projected.

Table 11. significance level of linear correlation

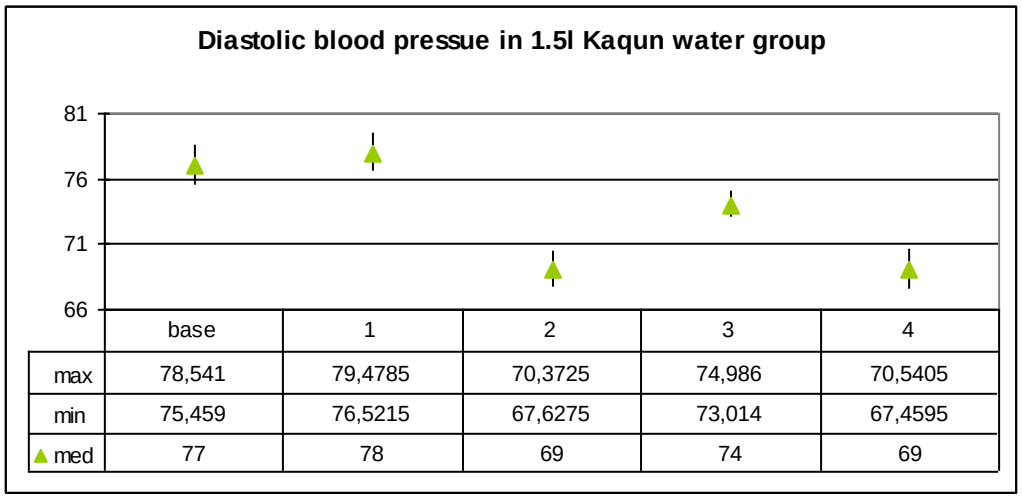
Pearson's linear correlation coefficient

	1	2	3	4
bázis	p=0,4836	p=0,8258	p=0,9102	p=0,3262
1		p=0,0986	p=0,7411	p=0,1351
2			p=0,0347	p=0,0418
3				p=0,0348
4				

(white: not significant, yellow p<0,05, green: p<0,01, blue: p<0,001)

Analysis of the linear correlation shows only the increase in the third week was significant and the decrease in the fourth week compared to the second and third week.

Graph 7. 1.5l/day standard error analysis



	base	1	2	3	4
max	78,541	79,4785	70,3725	74,986	70,5405
min	75,459	76,5215	67,6275	73,014	67,4595
▲ med	77	78	69	74	69

Analysis of the average values shows that the third measurement is outside the margin of the base value's standard error, the decrease is continuous. The big standard deviation of the base value is caused by a 100mmHG value we measured in one volunteer. At the end of the test period we measured 74 mmHg by this volunteer.

1l/day group analysis

Table 12. 1 l/day effect analysis

group	average	median	standard deviation	relative deviation	normality (p)
base	73	73	5,278	0,0723	74,2
1. week	74,6	72	9,934	0,33	
2. week	77,26667	77	11,88	0,154	
3. week	71,46667	72	9,87	0,138	
4. week	74,2	71	12,64	0,17	

When analyzing the **dependent variables** and the **stochastic homogeneity test** no significance could be found.

Table 13. Significance level of linear correlation

Pearson's linear correlation coefficient

	1	2	3	4
base	p=0,6214	p=0,5564	p=0,7856	p=0,3763
1		p=0,0014	p=0,0000	p=0,0006
2			p=0,0088	p=0,0080
3				p=0,0085
4				

The linear correlation analysis shows that the change compared to the base values is not significant; the internal significance however is expressed (increase, decrease respectively).

0.5l/day group analysis

Table 14. 0.5l/day effect analysis

group	average	median	standard deviation	relative deviation	normality (p)
base	78,53846	80	7,795462	0,0993	0,7375
1. week	77,53846	75	11,11767	0,143	0,8447
2. week	77,76923	78	6,326582	0,0814	0,9505
3. week	79,15385	79	8,706761	0,11	0,9752
4. week	76,15385	75	7,776526	0,102	0,9891

When analyzing the **dependent variables** and the **stochastic homogeneity test** no significance could be found.

Table 15. Significance level of linear correlation

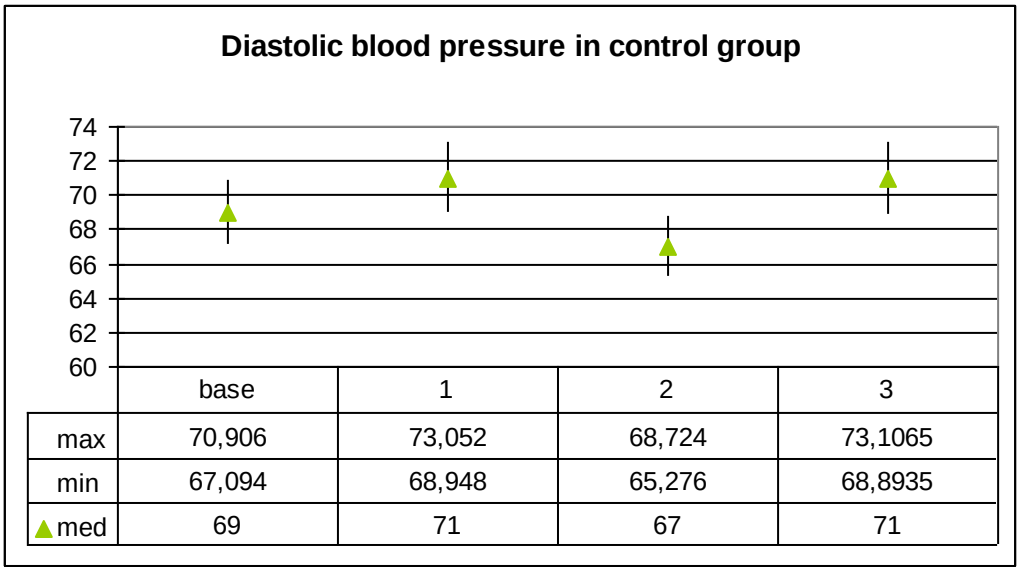
Pearson's linear correlation coefficient

	1	2	3	4
base	p=0,0069	p=0,1530	p=0,1888	p=0,3390
1		p=0,0550	p=0,0034	p=0,2198
2			p=0,1633	p=0,0562
3				p=0,4207
4				

The linear correlation analysis shows that the change compared to the base values is not significant.

Control group analysis

Graph 8. Control group diastolic values



The consumption of control water did not have a significant effect on the diastolic blood pressure values, the moves were within the margin of the standard error.

Overall only the consumption of 1.5l/day had a significant effect on the diastolic blood pressure.

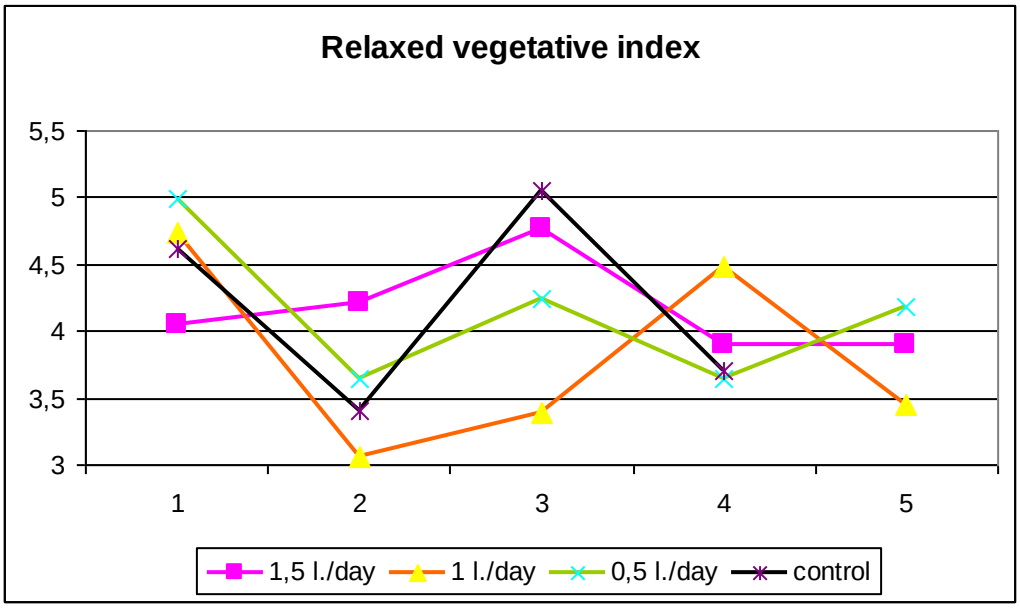
Relaxed vegetative index test

The vegetative index is the quotient of the average R-R distance divided by the standard deviation. The heart frequency is controlled by the autonomic nervous system, an immediate reaction can be seen to the body's physical and psychological effects. The hypothesis of the study is that the consumption of Kaqun water improves the body's cope with stress to physical impacts. Heart frequency is an immediate indicator of the body's physical and psychological effects. It indicates an external effect, that in the second week in every group (control included) increase was observed.

Table 16. Change in the relaxed vegetative index

Change of vegetative index median						start – end difference (max-min difference)
weeks	base	1	2	3	4	
1,5 l./day	4,05	4,22	4,76	3,9	3,9	-0,15 (-0,86)
1 l./day	4,74	3,06	3,39	4,48	3,45	-1,29 (-1,68)
0,5 l./day	4,99	3,65	4,25	3,65	4,18	-0,81 (-1,34)
control	4,62	3,4	5,05	3,71		-0,81 (+1,65)

Graph 9. Change of relaxed vegetative index



1.5l group analysis:

Table 17. 1.5l/day group change of vegetative index

group	average	median	standard deviation	relative deviation	normality (p)
base	4,4	4,05	1,629	0,37	0,6671
1. week	4,262727	4,22	1,356	0,318	0,8347
2. week	4,372727	4,12	1,903	0,435	0,9577
3. week	4,174545	3,9	1,184	0,284	0,6815
4. week	4,106364	3,9	1,582	0,385	0,8911

When analyzing the **dependent variables** and the **stochastic homogeneity test** no significance could be found.

Table 18. Significance level of linear correlation

Pearson's linear correlation coefficient

	1	2	3	4
base	p=0,4576	p=0,1763	p=0,7555	p=0,1970
1		p=0,0072	p=0,0267	p=0,0100
2			p=0,0291	p=0,0339
3				p=0,0378
4				

The linear correlation analysis shows that the change to the base value is not significant, the values increase after the first week of consumption, then a constant, significant decrease can be observed.

1l/day group analysis:

Table 19. 1l/day consumption data

group	average	median	standard deviation	relative deviation	normality (p)
base	4,753571	4,715	1,53	0,307	0,7449
1. week	3,176429	3,03	1,517	0,434	0,5176
2. week	3,307143	3,36	1,15	0,324	0,5731
3. week	4,352143	4,465	1,795	0,383	0,304
4. week	3,393571	3,37	1,634	0,438	0,027

In the first week measurement after the consumption of Kaqun water, the stress index decreased, then a constant increase was observed, which did not reach the base value, then it decreased again in the fourth week.

When testing the **dependent variables** the equality of averages were tested (analysis of variance $p<0.001$, robust analysis of variance with degrees of freedom correction, Geisser-Greenhouse analysis, Huynh-Feldt analysis), significance level $p<0.01$

Stochastic homogeneity test (Friedman test, analysis of variance by ranking numbers, robust analysis of variance with degrees of freedom correction, Geissel-Greenhouse analysis, Huynh-Feldt analysis), significance level $p<0.001$.

Table 20. Significance level of linear correlation

Pearson's linear correlation coefficient

	1	2	3	4
bázis	p=0,0270	p=0,0234	p=0,9962	p=0,0284
1		p=0,0002	p=0,4958	p=0,0000
2			p=0,1560	p=0,0006
3				p=0,3152
4				

The change is significant compared to the base values except for the third week. The change is significant compared to the first week.

0.5l/day group analysis:

Table 21. 0.5l/day group data

group	average	median	standard deviation	relative deviation	normality (p)
base	4,537692	4,99	1,056	0,233	0,1681
1. week	4,566154	3,65	3,238	0,709	0,2715
2. week	4,296923	4,25	1,226	0,285	0,7581
3. week	3,601538	3,65	1,008	0,28	0,9905
4. week	4,426154	4,18	1,874	0,423	0,839

The first week consumption did not have any effect, the decrease started from the second week, then it showed increase again at the last measurement.

When analyzing the **dependent variables** and the **stochastic homogeneity test** no significance could be found.

Table 22. 0.5 l/day significance level of linear correlation

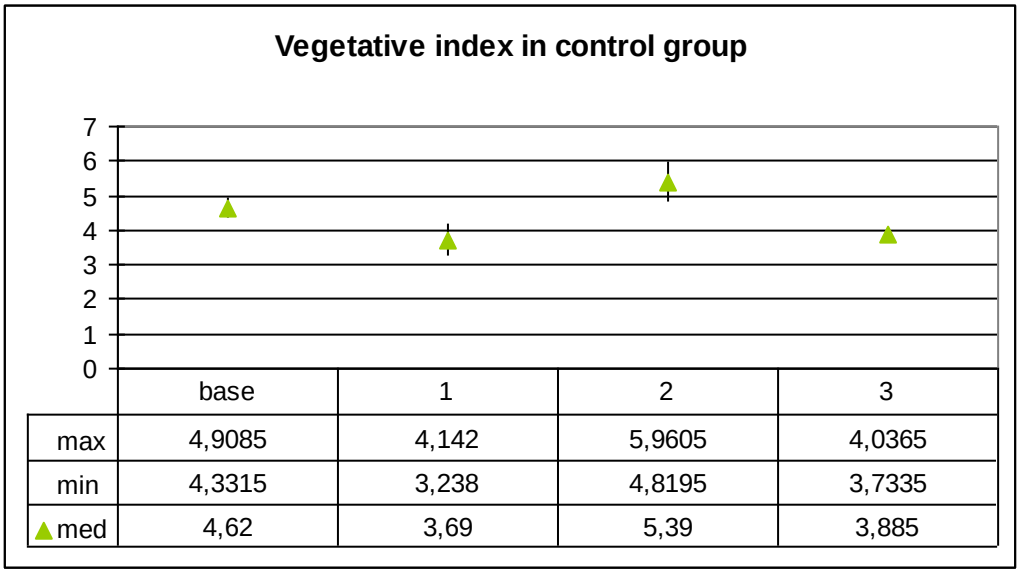
Pearson's linear correlation coefficient

	1	2	3	4
bázis	p=0,3771	p=0,8038	p=0,5253	p=0,1154
1		p=0,8662	p=0,8625	p=0,0344
2			p=0,1016	p=0,7563
3				p=0,5468
4				

No significance could be shown in the linear correlation test.

Control group analysis:

Graph 10. Change of vegetative index in control group



	base	1	2	3
max	4,9085	4,142	5,9605	4,0365
min	4,3315	3,238	4,8195	3,7335
▲med	4,62	3,69	5,39	3,885

The relaxed vegetative index shows an undulating run, but none of the changes is significant.

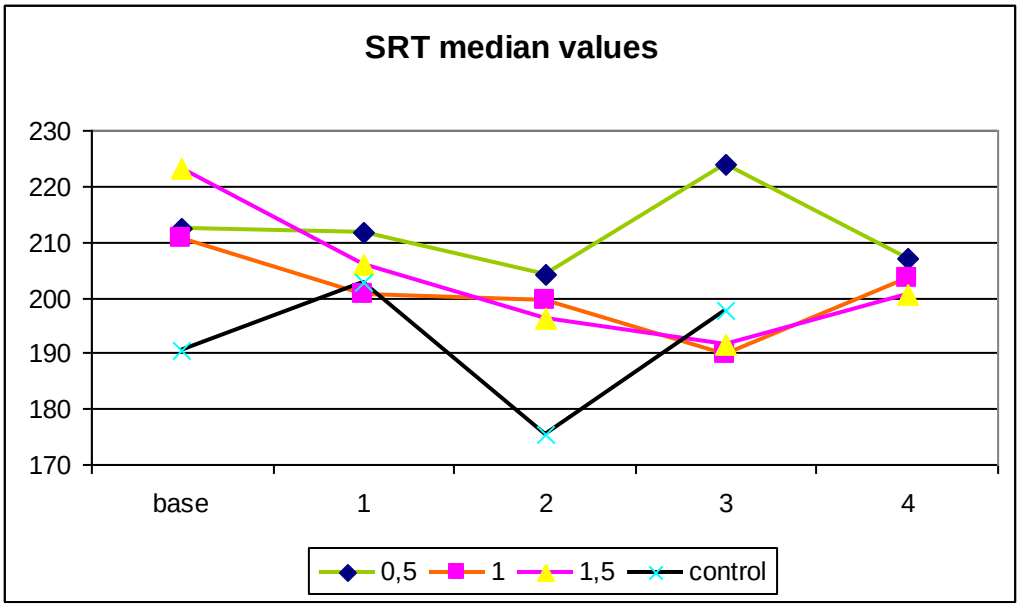
Summary:

The change of the relaxed vegetative index in the 1.5l group shows a significant increase from the second week onwards, while in the 1l group the decrease shows a constant significant value. Based on this the 1l group should be highlighted.

SRT analysis

The change in the reflex time indicates the speed of the nerve impulses. The measurement was done with classic method, push-button reply for an acoustic stimulus. The time between the sounds was random. We kept the lowest values, the three highest values were excluded.

Graph 11. Srt measurement values



In case of the raw data, visible change ban be seen in the 1.5l/day and 1l/day groups, in case of the smaller dose and control group it is unclear.

Table 23. Change of SRT values

	base	1	2	3	4
0,5	212,38	211,8445	204,207	224,0695	207,1035
1	210,741	200,483	199,517	189,793	203,429
1,5	223	205,897	196,36	191,429	200,571
control	190,625	202,824	175,5	197,667	

Due to the high standard deviation we decided to trim the values and did the evaluation after that.

1.5l group analysis:

Graph 12. Effect of 1.5l on SRT

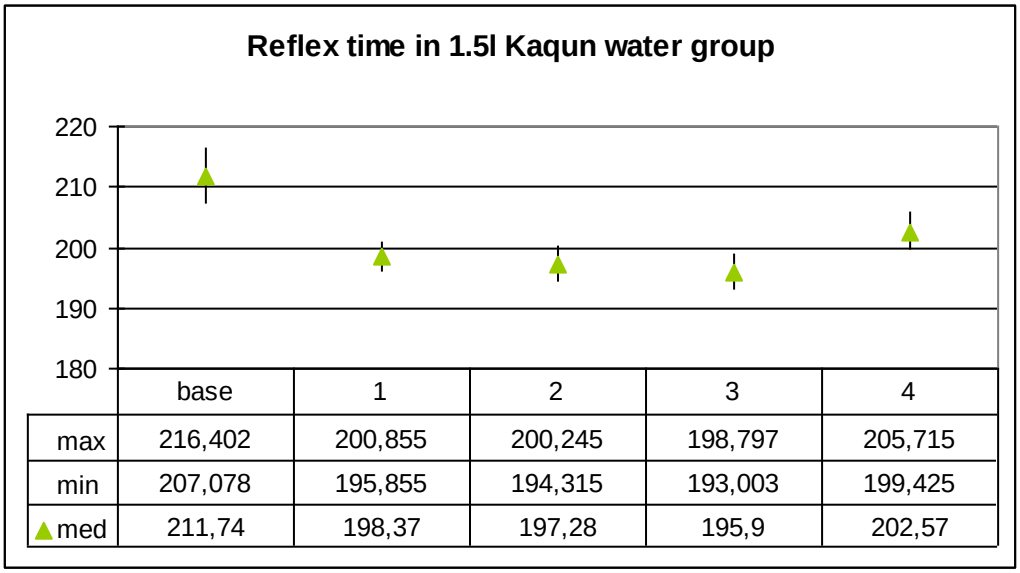


Table 24. Effect of 1.5l Kaqun water on SRT

group	average	median	standard deviation	relative deviation	normality (p)
base	211,74	210,621	27,97	0,132	0,913
1. week	198,37	201,172	15,09	0,0761	0,99
2. week	197,28	196,36	17,79	0,0902	0,9934
3. week	195,9	191,429	17,38	0,0887	0,9794
4. week	202,07	200,571	18,87	0,0934	0,9696

The reflex time constantly decreased until the fourth measure and there was a small increase at the end within the margin of error.

When testing the **dependent variables** the equality of averages were tested (analysis of variance $p<0.001$, robust analysis of variance with degrees of freedom correction, Geisser-Greenhouse analysis, Huynh-Feldt analysis), significance level $p<0.01$.

Stochastic homogeneity test (Friedman test, analysis of variance by ranking numbers, robust analysis of variance with degrees of freedom correction, Geissel-Greenhouse analysis, Huynh-Feldt analysis), significance level $p<0.001$.

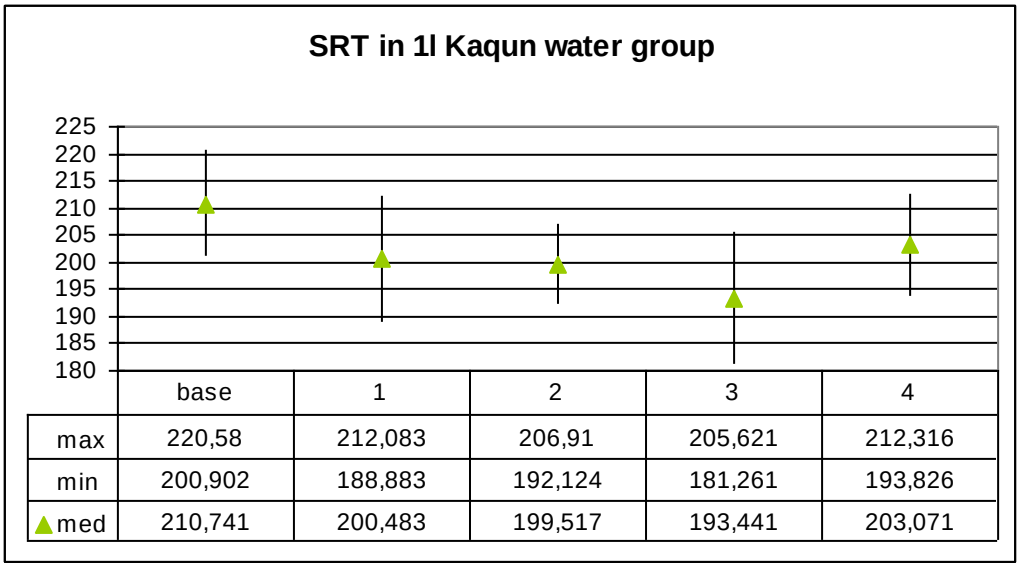
Table 25. Pearson's linear correlation coefficient

	1	2	3	4
bázis	p=0,0000	p=0,0005	p=0,0002	p=0,0003
1		p=0,0006	p=0,0000	p=0,0003
2			p=0,0000	p=0,0000
3				p=0,0000
4				

The data and analysis show a strong significance between the water consumption and the improvement in the reflex time.

1l/day group analysis:

Graph 13. Effect of 1l/day Kaqun water



	base	1	2	3	4
max	220,58	212,083	206,91	205,621	212,316
min	200,902	188,883	192,124	181,261	193,826
▲med	210,741	200,483	199,517	193,441	203,071

In the 1 liter dose there is a constant decrease in the median up until the fourth measurement, in the fifth measurement there is an increase within the margin of error.

Table 26. Effect of 1l/day on SRT

group	average	median	standard deviation	relative deviation	normality (p)
base	208,0353	210,741	35,47	0,171	0,9013
1. week	209,4009	200,483	41,83	0,2	0,7479
2. week	205,6957	199,517	26,66	0,13	0,9732
3. week	207,1317	193,441	43,91	0,212	0,8896
4. week	210,7258	203,071	33,33	0,158	0,6636

The equality of averages and the stochastic homogeneity test does not show any significance.

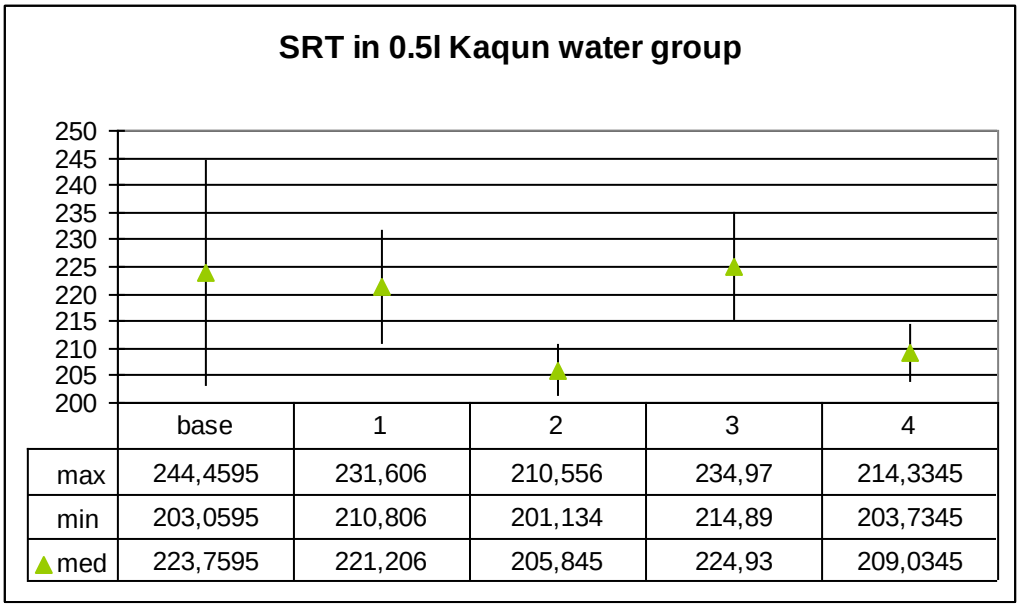
Table 27. Pearson's linear correlation coefficient

1	2	3	4
p=0,0000	p=0,0000	p=0,0000	p=0,0000
	p=0,0000	p=0,0000	p=0,0000
		p=0,0000	p=0,0000
			p=0,0000

The linear correlation analysis shows a big significance in the values.

0.5l/day group analysis:

Graph 14. 0.5 l/day effect on SRT



	base	1	2	3	4
max	244,4595	231,606	210,556	234,97	214,3345
min	203,0595	210,806	201,134	214,89	203,7345
▲med	223,7595	221,206	205,845	224,93	209,0345

The base value has a high standard deviation due to a 403 value, so the standard error is also high. To the second and third measurement the value of the standard error also decreased significantly, which resulted in a decrease of the standard deviation in the test subjects.

Table 28. Effect of 0,5l Kaqun water on SRT

group	average	median	standard deviation	relative deviation	normality (p)
base	233,44	223,76	65,44	0,28	0,5082
1. week	219,12	221,21	32,88	0,15	0,9182
2. week	208,27	205,84	14,9	0,0715	0,7599
3. week	223,83	224,93	31,74	0,142	0,9208
4. week	210,99	209,03	16,76	0,0794	0,9978

The equality of averages and stochastic homogeneity test do not show any significance.

Table 29. Pearson's linear correlation coefficient

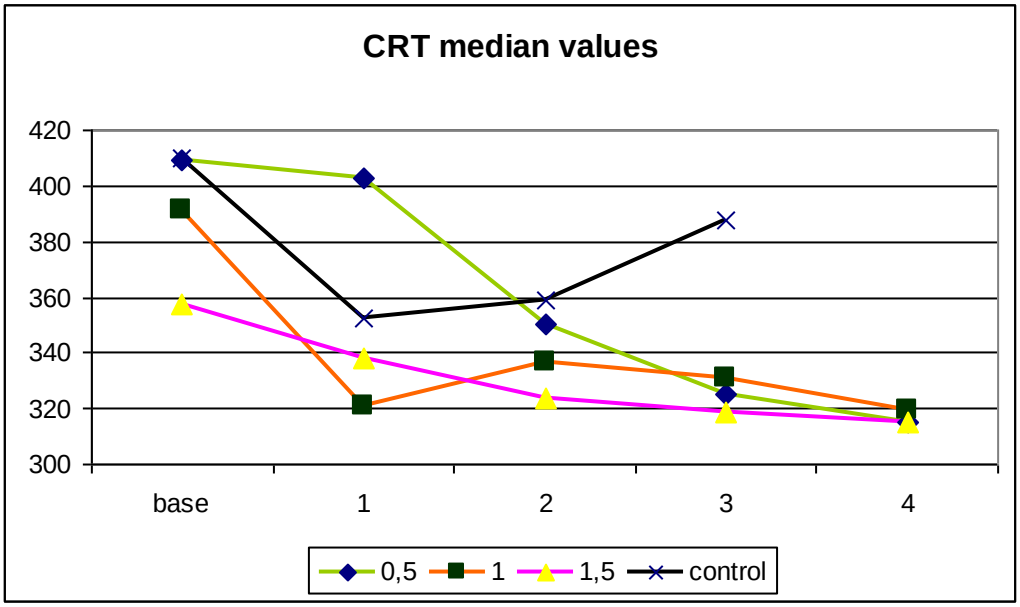
1	2	3	4
p=0,0005	p=0,0093	p=0,0038	p=0,0045
	p=0,0002	p=0,0000	p=0,0000
		p=0,0005	p=0,0000
			p=0,0000

The linear correlation test shows a strong significance.

Cognitive reaction time

The time requirement for the cognitive processes measures the usage time of the work memory besides divided attention.

Graph 15. CRT test results



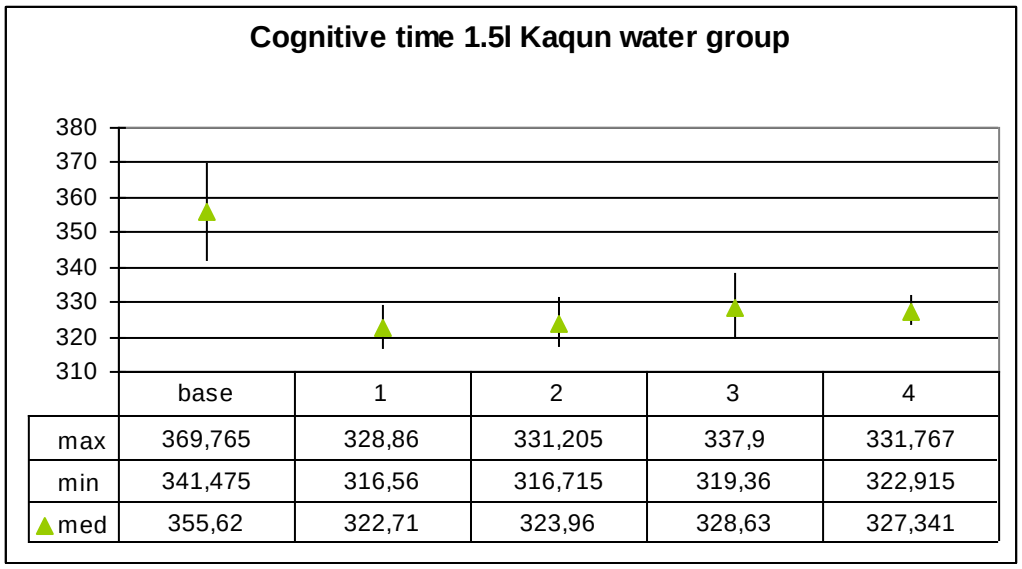
We can see that related to the base time a significant acceleration can be seen compared to the control group.

Table 30. Change of CRT values

	base	1	2	3	4
0,5	409,2105	402,444	350,493	324,999	315,149
1	391,2	320,708	336,429	330,586	319,583
1,5	357,731	338,069	323,962	318,655	315,321
control	410,101	352,692	359,269	387,333	

1.5l group analysis

Graph 16. Effect of 1.5l on CRT

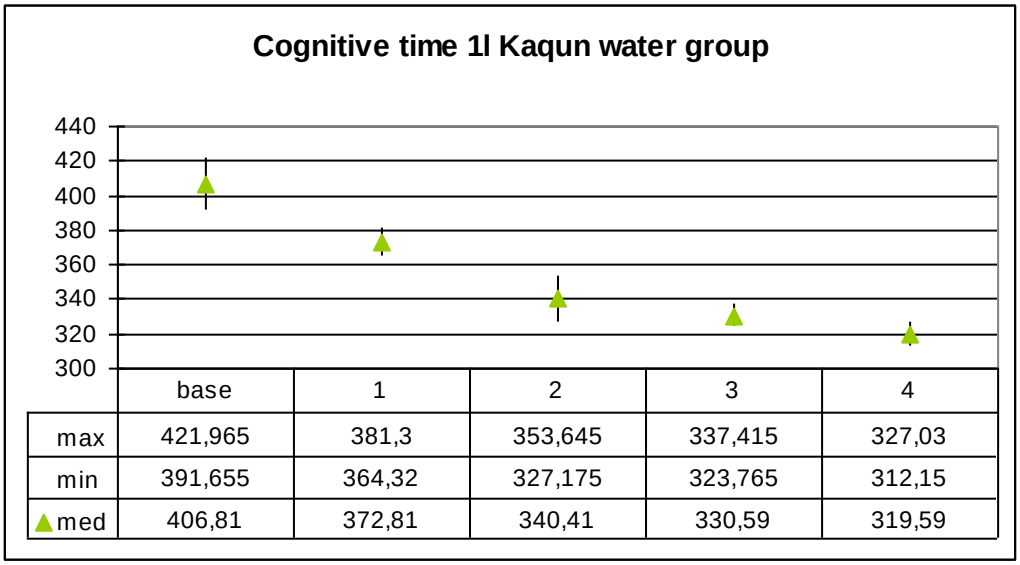


	base	1	2	3	4
max	369,765	328,86	331,205	337,9	331,767
min	341,475	316,56	316,715	319,36	322,915
▲med	355,62	322,71	323,96	328,63	327,341

The consumption of 1.5l Kaqun water shows a significant change in the first week compared to the base value in case of both Pearson's linear correlation coefficient (p=0.0276), and Wilson's robust correlation coefficient (p=0.0399). Changes in the subsequent weeks are minimal, significance can not be detected.

1l/ group analysis

Graph 17. Effect of 1l on cognitive processes

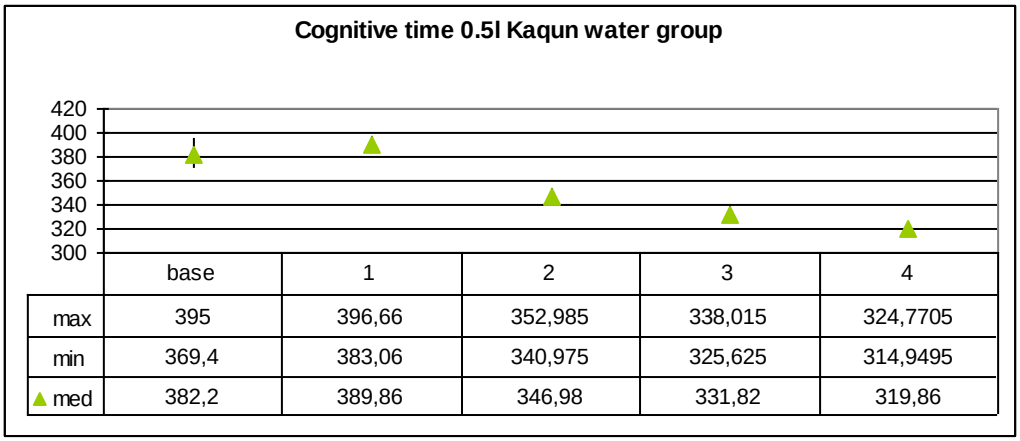


	base	1	2	3	4
max	421,965	381,3	353,645	337,415	327,03
min	391,655	364,32	327,175	323,765	312,15
▲med	406,81	372,81	340,41	330,59	319,59

The decrease is constant compared to the base value. Both the dependent variables and the stochastic homogeneity test showed strong significance. The linear regression test showed significance in changes compared to the base value.

0.5l group analysis

Graph 18. Effect of 0.5l Kaqun water on cognitive effects



	base	1	2	3	4
max	395	396,66	352,985	338,015	324,7705
min	369,4	383,06	340,975	325,625	314,9495
▲med	382,2	389,86	346,98	331,82	319,86

The median value increased in the first week compared to the base value, then a constant decrease followed. From the dependent variables the equality of averages and the stochastic homogeneity test showed significance. The linear regression test showed significance in changes compared to the first week.

Change in oxygen saturation

The consumption of water with higher oxygen content should increase oxygen saturation and improve the body's oxygen supply.

Graph 19. Change of oxygen saturation

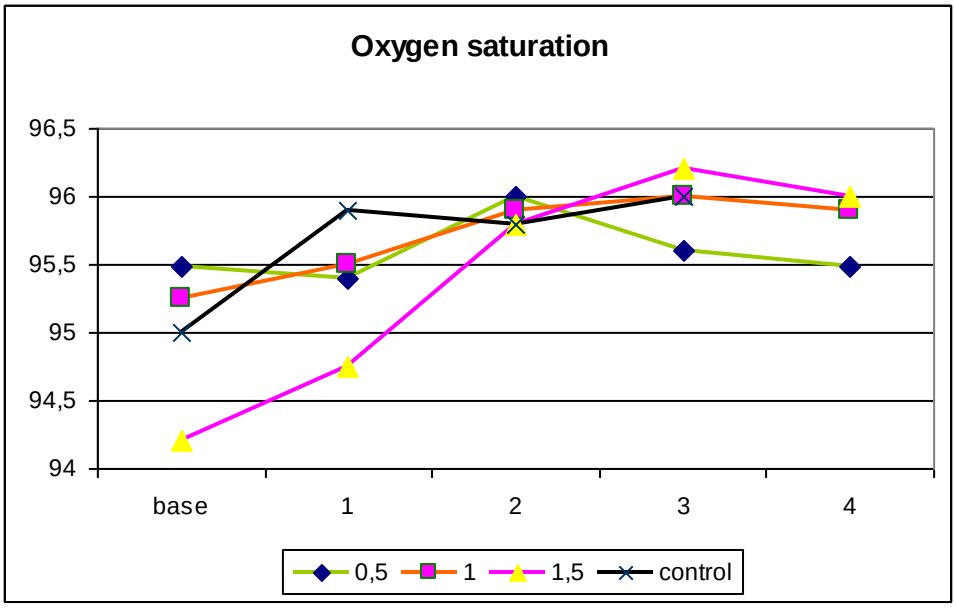
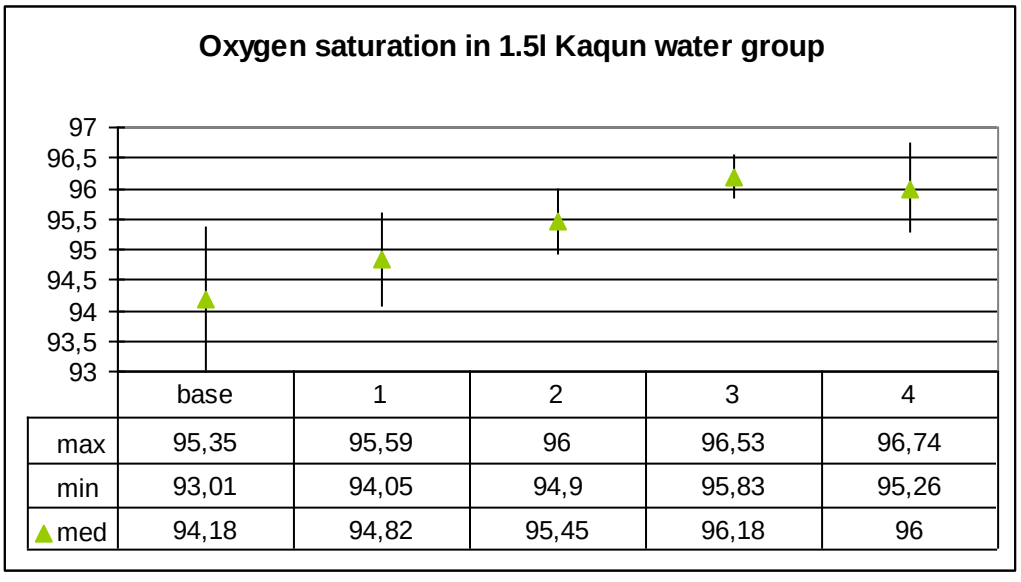


Table 31. Change of oxygen saturation

	1,5 l	1 l	0,5 l	control
Change in %	2	0,73	0,54	1

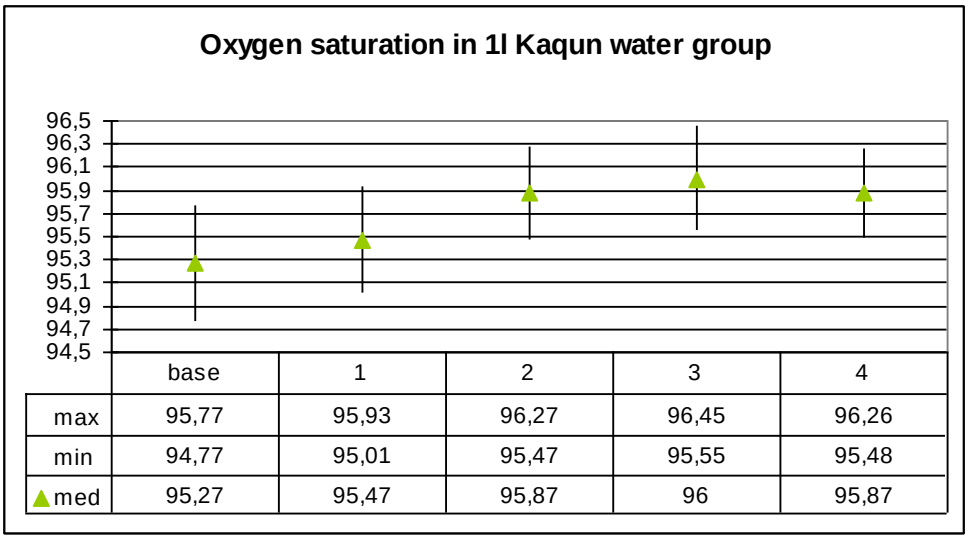
The increase in saturation in the control group was 1%. The 1% increase is probably due to the water bodies being filled up. In comparison, we observed saturation increase in the 1l and 1.5l groups. The linear correlation test showed significant changes in both the 1.5l and 1l groups.

Graph 20. Change of oxygen saturation 1.5 l/day



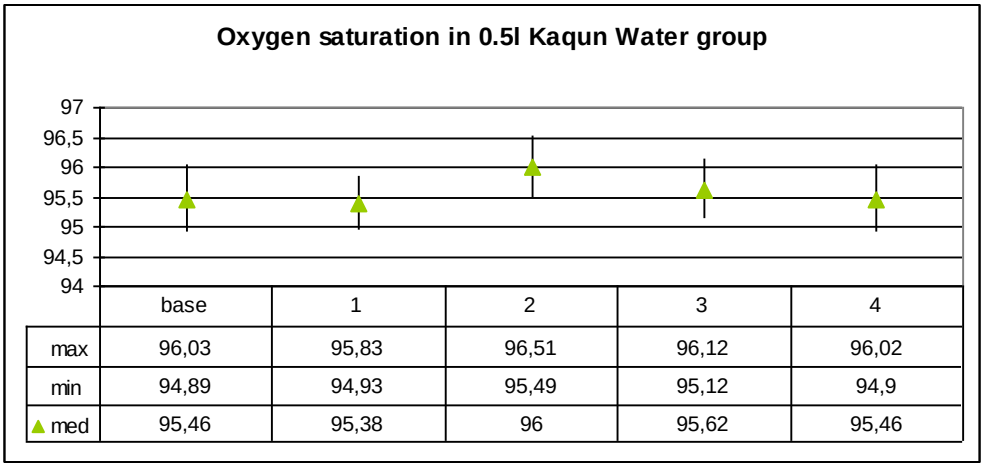
	base	1	2	3	4
max	95,35	95,59	96	96,53	96,74
min	93,01	94,05	94,9	95,83	95,26
▲med	94,18	94,82	95,45	96,18	96

Graph 21. Change of oxygen saturation 1 l/day



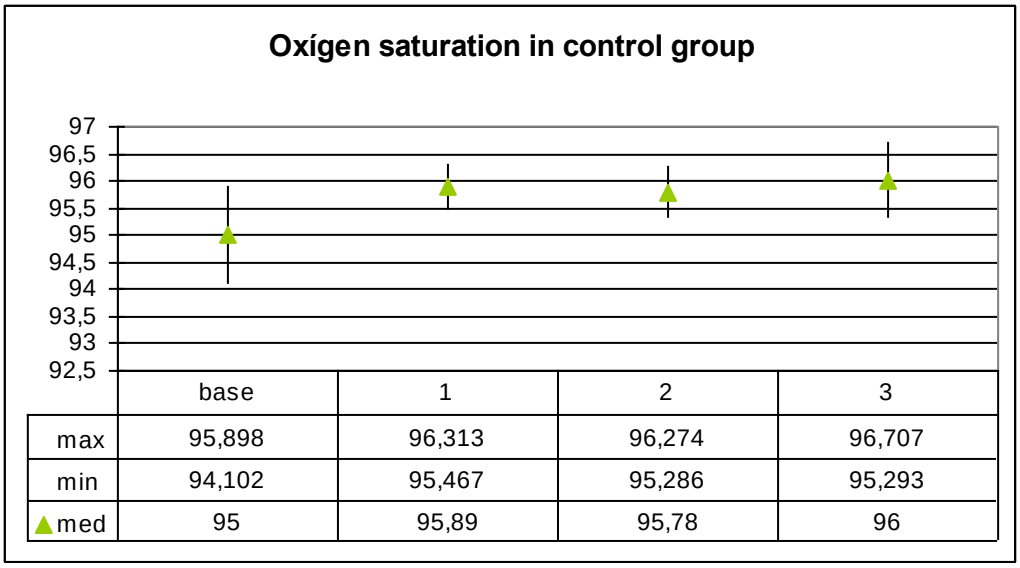
	base	1	2	3	4
max	95,77	95,93	96,27	96,45	96,26
min	94,77	95,01	95,47	95,55	95,48
▲med	95,27	95,47	95,87	96	95,87

Graph 22. Change of oxygen saturation 0.5 l/day



	base	1	2	3	4
max	96,03	95,83	96,51	96,12	96,02
min	94,89	94,93	95,49	95,12	94,9
▲med	95,46	95,38	96	95,62	95,46

Graph 23. Change of oxygen saturation control group



	base	1	2	3
max	95,898	96,313	96,274	96,707
min	94,102	95,467	95,286	95,293
▲med	95	95,89	95,78	96

Dosage and efficacy and maximum time of effect appearance

An important question is in what dosage should the water be consumed and when does the maximum impact appear at given dosage.

Table 32. Evaluating the efficacy:

	sist. RR	diast. RR	veg. index	SRT	CRT	saturation	total points
1,5 l	2	1	3	1	3	1	11
1 l	1	3	1	2	2	2	13
0,5 l	2	2	2	3	1	3	13

We put in this table depending on the scale of changes first, second or third place. From this we can prepare the dosage suggestions. So:

Consumption of 0.5l daily is recommended to increase the CRT.

Consumption of 1l daily is recommended to decrease systolic blood pressure and reduce stress sensitivity.

Consumption of 1.5l daily is recommended for other cases.

The appearance of maximum impact generally falls on the third week in case of both the 1.5l and 1l dosage, then the values decrease. The exception in the cognitive time but even here the difference between the third and fourth week is minimal. Therefore basically the three week consumption followed by a one week break is the recommended dosage.

Completed domestic so far made with Kaqun water:

1. Katalin Pál dr.: Effect of oxygen-enriched water on tumor cells. 2004
2. Semmelweis University, Faculty of Physical Education and Sports: Effect of high oxygen content Kaqun water drink therapy and Kaqun bath therapy on psycho-physiological parameters 2007.
3. Hungarian Academy of Sciences Isotopes Research Institute, Department of Surface chemistry and Catalysts: Report on assessing the role of Kaqun water with high oxygen content on formation of reactive oxygen radicals in in vitro system. 2009.
4. National Institute of Chemical Safety: Effect of Kaqun water on immunological parameters of healthy volunteers. 2009.
5. National Institute of Chemical Safety, Department of Chemical Safety Research, Department of Molecular and Cell Biology: Citotoxicity study on Kaqun water. 2010.
6. National Institute of Chemical Safety, Department of Chemical Safety Research, Department of Molecular and Cell Biology: Examination of the antioxidant capacity influencing effect of Kaqun water. 2011.

KAQUN

Therapeutic effect by a mistletoe extract, Iscador and oxygen enriched water (Kaqun) on the development of experimental tumor (A2780, KB-3-1) growth in a scid mice model

Introduction

Iscador

Iscador, an extract from the semi-parasitic plant Viscum album, was found to inhibit 20-methylcholanthrene-induced carcinogenesis in mice. Intraperitoneal administration of Iscador (1 mg/dose) twice weekly for 15 weeks could completely inhibit 20-methylcholanthrene-induced sarcoma in mice and protect these animals from tumour-induced death. Iscador was found to be effective even at lowered doses. After administration of 0.166, 0.0166 and 0.00166 mg/dose 67, 50 and 17% of animals respectively did not develop sarcoma xx (Kuttan G, Menon LG, Kuttan R.Prevention of 20-methylcholanthrene-induced sarcoma by a mistletoe extract, Iscador.; 1996 May;17(5):1107-9.) In human peripheral blood mononuclear cells stimulated by Viscum album L. ssp. album the levels of CD4(+)CD25(+) and CD8(+)CD25(+) T cells, CD69 expressions in the activated T lymphocytes and CD3(-)CD16(+)CD56(+) NK cells increased compared to the cells that were not stimulated by this herbal. xx (Fidan I, Ozkan S, Gurbuz I, Yesilyurt E, Erdal B, Yolbakan S, Imir T.:The efficiency of Viscum album ssp. album and Hypericum perforatum on human immune cells in vitro. Immunopharmacol Immunotoxicol. 2008;30(3):519-28.) There are many publications on the therapeutical effect of Iscador regarding human tumors, but Iscador is still one of the alternative therapies.

KAQUN

Kaqun is a special water with high oxygen content, suitable for use in the form of drinking water or bathing. As a result of the KAQUN technology oxygen is present in a stable form with a concentration of 18-25 mg/l, which is manifold of the oxygen content of normal drinking water.

Killing of cancer cells by macrophages in a non-ADCC manner via apoptosis is induced by reactive oxygen species (ROS). (Hicks AM, Willingham MC, Du W, Pang CS, Old LJ, Cui Z: Effector mechanisms of the anti-cancer immune responses of macrophages in SR/CR mice. Cancer Immun. 2006 Oct 31;6:11). It was also observed, that target cell antioxidant mechanisms play an important role in the outcome of the cytotoxic response of human polymorphonuclear leukocytes (PMN) against red blood cells (RBC) and K562 tumor cells (van Kessel KP, van Strijp JA, van Kats-Renaud HJ, Miltenburg LA, Fluit AC, Verhoef J.: Uncoupling of oxidative and non-oxidative mechanisms in human granulocyte-mediated cytotoxicity: use of cytoplasts and cells from chronic granulomatous disease patient. J Leukoc Biol. 1990 Oct;48(4):359-66).

Kaqun water has a significant influence on grow of tumor cells in vitro, although there are great differences between its effect on different cell lines. It has no influence on Hep G2 (human hepatocellular carcinoma) cells (02-ctox-10, OKBI-KBKF Budapest, 2010.12.20), while it significantly decreases the growth of LLT-HH (highly metastatic variant of Lewis lung carcinoma) and H59 (Lewis Lung Carcinoma Variant) cell lines in a dose dependent manner (). Furthermore human experiences suggest a benefit effect of kanqun water on several human tumors (including ovarian carcinoma) (xx)

(http://kaqun.hu/en/scientific-results/12-az-orszagos-kemiai-bizottsagi-intezet-jelentese-2009)

Based on the above knowledge, the supposed therapeutically influence of the mistletoe extract, Iscador and that of the oxygen enriched water (Kaqun) was investigated in a scid mice model using human cancer cell lines (cervical carcinoma cell line KB-3-1, ovarian cancer cell line A2780).

Material and methods

Animal Study

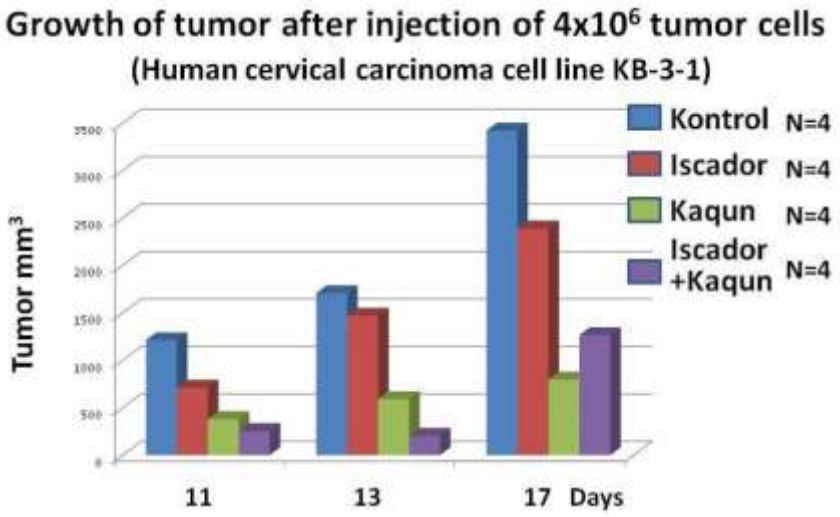
CB17/ICR SCID (severe combined immunodeficient) mice, approximately xx weeks of age and weighing approximately 20 g, were used to establish orthotopic cervical carcinoma and ovarian carcinoma tumors for the experiment. All these mice were bred in the Laboratory Animal Facility at xx, were maintained in specific pathogen-free conditions, and received commercial food and water ad libitum. Institution guidelines were followed in handling the animals. To establish the orthotopic tumors, cultured A2780 and KB-3-1 were harvested with 0.05% trypsin-EDTA (GIBCO BRL), washed in PBS, and resuspended in RPMI-1640 complete medium at xx 40 × 10^6 cells per milliliter.

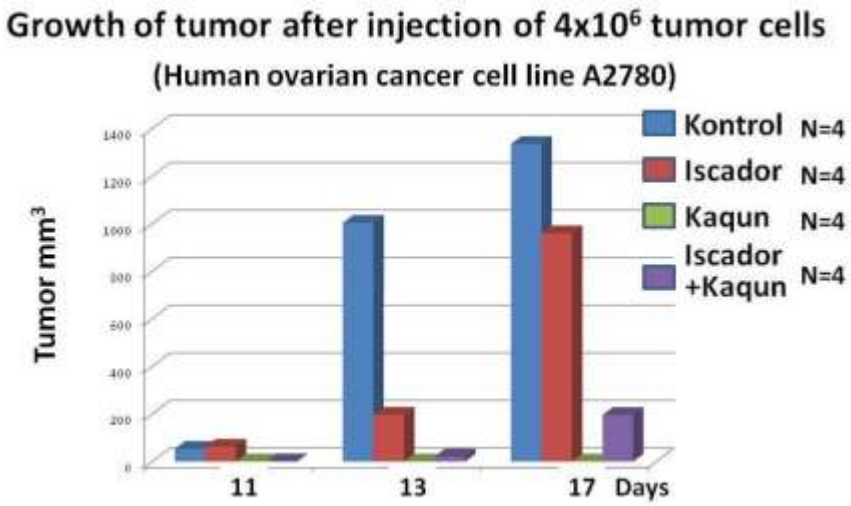
Approximately xx ml of the cell suspension (about 4 × 10^6 cells) was subepidermaly injected into the leg of the animal, while xx ml of the cell suspension (about 2 × 10^6 cells) into the back of the mice. Tumor grow was measured weekly twice. Four animals one of each group were sacrificed xx days after implantation, and further 4 after xx days. The tumors were removed, embedded in paraffin, or liquid nitrogen and sectioned for histopathologic analysis.

Iscador 1 ng/kg

Results

Eleven days after injection of tumor cells, the cervical carcinoma cell line, resulted well defined tumors in all groups. At that time ovarium carcinoma cells resulted detectable tumor only in the control group and in the iscador treated group.





Discussion

KAQUN

As a result of the KAQUN technology oxygen is present in a stable form with a concentration of 18-25 mg/l, which is manifold of the oxygen content of normal drinking water.

30 examined persons participated in a 21 day bathing and water drinking treatment. The participants bathed once a day in the morning in individual bathtubs filled with 37 oC water containing stable oxygen, for a maximum of 50 minutes per occasion. The water drinking cure consisted of drinking 1.5 liter Kaqun drinking water every day in parallel with the baths. The bathing cure followed the standards established in the Kaqun Health Program Service

The percentage of NK-cells increased significantly after the second week of the treatment both for the whole group and for women. In men an increase was observed, but due to the large deviations in individual results, the change was not significant statistically. Individually, in general either there was no change or an increase was observed during the three weeks of the study. (The effect of KAQUN-water on the immune parameters of healthy volunteers (http://kaqun.hu/en/scientific-results/12-az-orszagos-kemiai-bizottsagi-intezet-jelentese-2009)

The uptake of tumor xenograft can be influenced (decreased) by treatment with Iscador + KAQUN water? (mice will drink normal or KAQUN water and will be treated with Iscador ip. weekly twice)

Investigations are needed to determine the patomechanism behind the tumor growth inhibitory effect of Iscador, Kaqun and Iscador+Kaqun therapy.

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The effects of KAQUN-water on patients undergoing oncology treatment –

a randomized study

Dr. Gabriella Liszkay - National Institute of Oncology

Kaqun Hungária Ltd.

Kaqun is a special type of high oxygen-content water, which can be used as water for therapy by drinking and/or bathing.

Observations: improves fitness and well-being, successful in wound treatment, reduces skin inflammation. Immunological examinations showed that percentage ratio of NK cells increased, the expression of CD25 surface marker was influenced, increased reactive oxygen intermediates' production improves activity of neutrophil granulocytes.

Aim: to study the effects of Kaqun-water on patients undergoing oncological treatment.

Primary endpoints: radiation-induced dermatitis, drug-induced dermatitis, fitness and state of mind, studying changes in exulceration.

Secondary endpoints: changes in cutaneous manifestations of melanoma, tumor response in complex oncotherapy of stage IV melanoma.

Patient groups:

Group 1: 10 randomly chosen patients, with any grade of skin symptoms due to targeted therapy, undergoing any dermatological treatment;

Group 2: 10 randomly chosen patients with solid tumors having gone under radiotherapy (any radiation source);

Group 3: 5 patients that were not treated with radiotherapy due to cutaneous metastases (intransit) of malignant melanoma;

Group 4: 10 patients undergoing complex oncotherapy for stage IV malignant melanoma.

Our preliminary results show, that the Kaqun-water has a favorable effect on the patient's fitness and psychological status, and on the level of side effects of oncotherapy.