

## 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 therapeutic 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 growth 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 kaqun 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 therapeutic 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  $40 \times 10^6$  cells per milliliter.

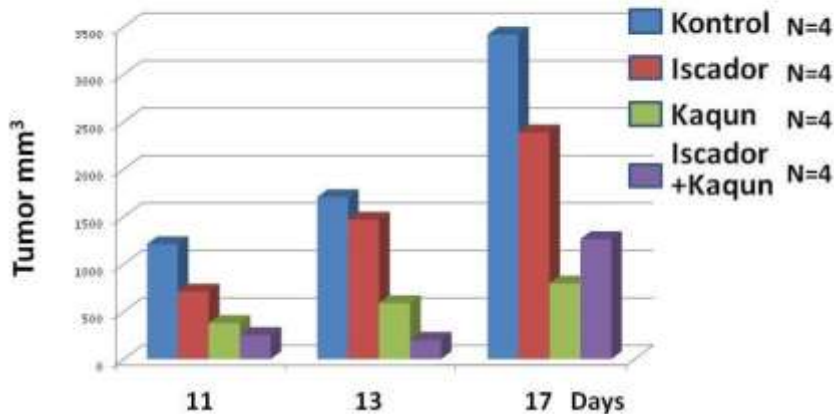
Approximately xx ml of the cell suspension (about  $4 \times 10^6$  cells) was subepidermally injected into the leg of the animal, while xx ml of the cell suspension (about  $2 \times 10^6$  cells) into the back of the mice. Tumor growth was measured weekly twice. Four animals of each group were sacrificed 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

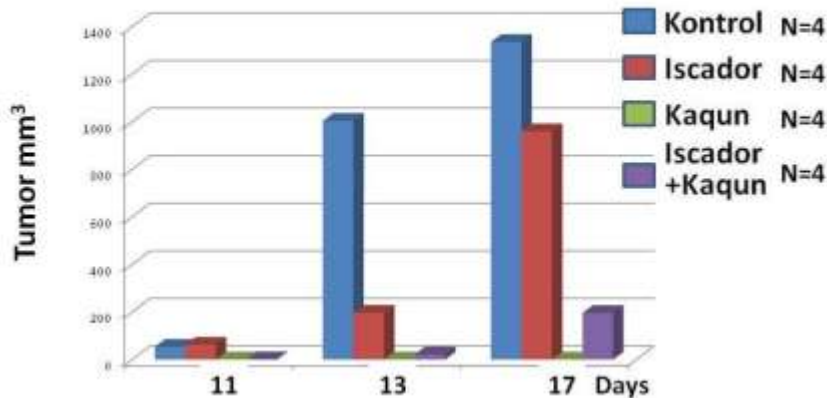
## Results

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

### Growth of tumor after injection of $4 \times 10^6$ tumor cells (Human cervical carcinoma cell line KB-3-1)



### Growth of tumor after injection of $4 \times 10^6$ tumor cells (Human ovarian cancer cell line A2780)



## Discussion

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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.