Investigations on the beneficial effects of Bormia-Water© with cultured cells

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Background and question of this study

Water is a tasteless, odorless, and nearly colorless chemical substance that is the main constituent of most living organisms. From the strictly academic point of view, water is just H_2O and exists in three different phases, namly liquid state, solid state and gaseous state. Water posesses a number of unique properties which can be explained by natural science only in part.

From the metaphysical point of view, water is much more than just hydrating the body. When water is flowing in spirals and vortices and is continuously changing its state, the molecules organise themselves into structures, layers or clusters of energy which carry constructive information in form of vibration energy like a magnetic tape. If these are beneficial, they may be able to restore healthy resonance in the human body, as in homoeopathy. For more details, see Refs. 1-3.

In the present scientific study, current cell biological methods were used to investigate whether tap water, after passing through a single device from Bormia in form of rhythmic, left and right spinning spirals and an additional crystal waterfall (a device of stones and crystals like in a creek) has received and stored vibrational energy to possess beneficial health effects in direct comparison to the same tap water without using the Bormia device.

Bormia product & tested waters

The Bormia Ananda 20 GS with gold/silver-coated, rhythmically left and right spinning spirals and with an additional crystal waterfall was used to improve the initial tap water in the investigations shown here in detail. The water used for the tests was either tap water from D-83052 Bruckmühl or from D-88696 Owingen, each without filtration but nevertheless damaged by pressure. For further informations on the product, see www.bormia.de and www.zero-point-energy-water.com.

Examination of the antioxidant effect in a cell-free test system

In this cell-free test system, it was tested whether Bormia-Water© is able to inactivate free oxygen radicals better than the initial tap water and, thus, prevent oxidative stress. The different concentrations of the two test waters, WST-1 (a water-soluble tetrazolium dye) were mixed. The reaction was started by the addition of potassium peroxide in Aqua dest. (1 mg/ml) and the superoxide anion radicals in the reaction mixture, which were not inactivated by the test water caused a cleavage of the dye which was accompanied by a change in the optical density (= colour). The optical density was continuously recorded as a differential measurement ΔOD = 450 – 690 nm on the Elisa reader (BioTekSLx808) and, after linear regression of the reaction curves, was evaluated in the form of the slope (ΔOD per min) and compared between the two test waters. The relative data for Bormia-Water© in comparison to the tap water was finally evaluated.

As depicted in Fig. 1A, Bormia-Water© increased the antioxidant efficacy by an average of about 15 % and a maximum of 20 % when compared with the initial tap water. As a matter of fact, more free reactive oxygen radicals can be inactivated so that the body's own mechanisms to neutralize reactive oxygen radicals are strengthened when Bormia-Water© is taken regularly.

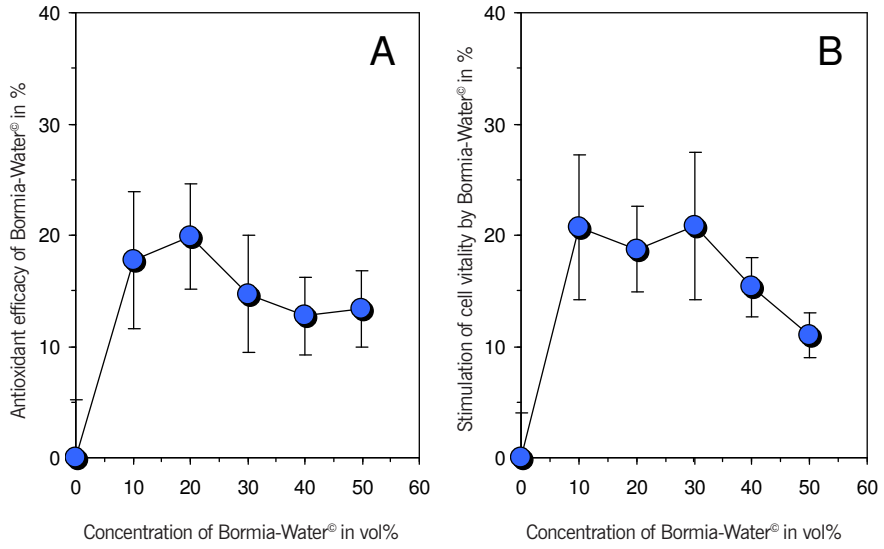

Fig. 1: Relative increase of the antioxidant effect (A) and vitality of cultured connective tissue cells (B) by Bormia-Water© in comparison to untreated initial tap water without flow (here set as 0). At all test concentrations, the increase by Bormia-Water© is statistically significant ($p < 0.01$; Wilcoxon-Mann-Whitney-Test). Data represent mean value ± standard deviation of 3 experiments.

Cell cultures of connective tissue cells

The investigations were performed with connective tissue fibroblasts of cell line L-929 (ACC-2; Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) and used in the subcultivation stages (passages) 50 to 52. Cells were routinely grown in RPMI 1640 with 10% foetal calf serum and 0.5 % of gentamycin and incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

Examination of cell vitality

For the experiments, the cells were seeded from mass cultures at a cell density of 20,000 cells/well in 96-well plates (200 µl culture medium/well) and incubated for 24 h to achieve cell attachment and metabolism.

Then, medium was changed and the different water concentrations of both waters were added. After 24 h of continuous exposure, culture medium was aspirated and replaced by 180 µl of fresh culture medium containing 20 µl XTT. The optical density of each well was measured after

0 min and after 120 min at 37°C as a differential measurement at ΔOD = 450 – 690 nm on the Elisa reader (BioTEK Elx 808) and compared between the two test waters. The relative data for Bormia-Water© in comparison to the tap water was finally calculated.

XTT is the sodium salt of 2,3-bis[2-methoxy-4-nitro-5-sulfopheny]-2H-tetrazolium-5-carboxyanilide and has a yellowish colour. Mitochondrial dehydrogenases of living cells cleave the tetrazolium ring of XTT and orange stained and water-soluble formazan crystals are formed. The intensity of the orange colour of the reaction solution is proportional to the cell vitality and is determined photometrically.

When compared to the untreated tap water without flow, Bormia-Water© caused a statistically significant increase in cell vitality by about 20% ($p < 0.01$; Wilcoxon-Mann-Whitney-Test; Fig. 1B). Although this increase in cell vitality was reduced at concentrations > 30 vol%, it was still significantly higher than the initial tap water. This stimulation in cell vitality might cause a higher physical performance and an improved well-being in humans.

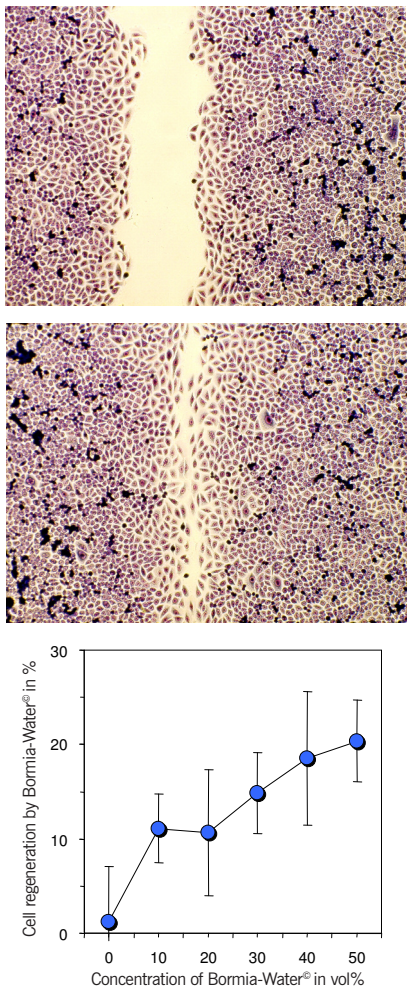

Fig. 2: Micrographs of cultured connective tissue cells to illustrate cell regeneration. (Upper picture) Migration of cells into a cell-free space within 2 hours after removal of the silicone insert and (middle picture) after 24 hours of continuous incubation with extensive recolonisation of the cell-free space. (Lower picture) Stimulation of cell regeneration by Bormia-Water© compared to untreated initial tap water (here set as 0). At a Bormia-Water© concentration ≥ 30 vol% the increase becomes statistically significant ($p < 0.01$; Wilcoxon Mann-Whitney test). Data represent mean value ± standard deviation of 3 experiments.

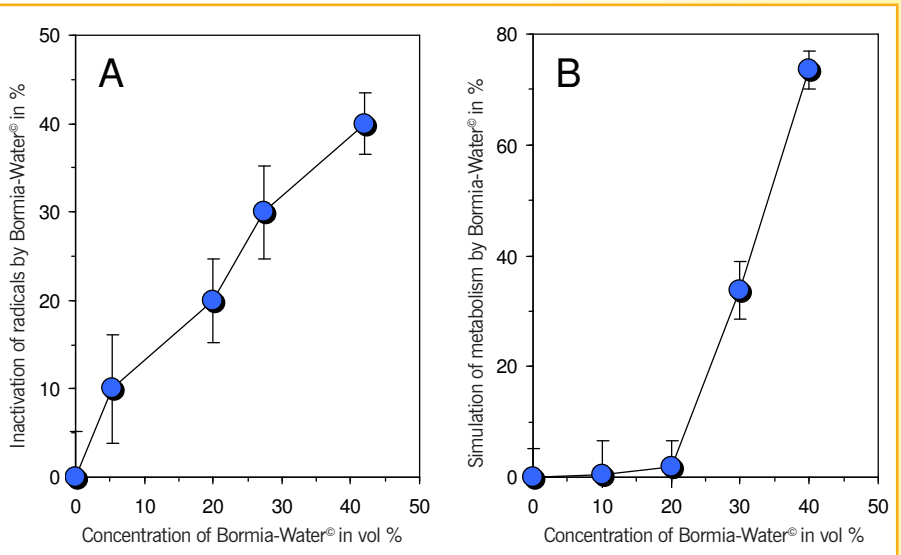

Fig. 3: (A) Relative inactivation of endogenously generated superoxide anion radicals by Bormia-Water© in comparison to the initial tap water without flow (here set as 0). At all concentrations of Bormia-Water© ≥ 20 vol% the increased inactivation is statistically significant ($p < 0.01$; Wilcoxon-Mann-Whitney test). Data represent mean value ± standard deviation of 3 experiments. (B) Relative stimulation of the energy metabolism of adherent HL-60 cells within 180 min after application of Bormia-Water© in comparison to the initial tap water without flow (here set as 0). Activation is statistically significant at all concentrations of Bormia-Water© > 20 vol% ($p < 0.01$; Wilcoxon-Mann-Whitney test). Data represent mean value ± standard deviation of 3 experiments.

Examination of cell regeneration

An improvement in cell vitality is usually coupled with a stimulation of cell regeneration. In this process, proliferation and migration of the cells is a key event at the cellular level. In order to investigate whether Bormia-Water© can also stimulate cell regeneration, the cells were seeded at a density of 50,000 cells/ml into the three cell culture compartments of silicone Culture-Insert 3 Wells (ibidi, Martinsried/Munich, Germany) which have been carefully placed on the surfaces in each well of a 12-well plate. The cell culture reservoirs are separated by a 500 µm thick wall. Due to their especially designed bottom, the inserts sticked to the surface firmly and completely prevented any cell growth under the walls.

Cells were cultured until a dense cell layer was achieved and the inserts were removed to leave sharp cell-free gaps (artificial wounds) without any cells. Cells began to migrate and proliferate into the cell-free space in order to close the wound (Fig. 2). After 24 hours, cells were fixed with methanol and stained according to Coomassie-Giemsa, air-dried and the width of the remaining wound was measured. For each test concentration of the waters 18 measurements were performed in triplicate experiments and the relative cell regeneration in comparison to the untreated tap water was calculated.

As depicted in Fig. 2, Bormia-Water© showed a dose-dependent stimulation of cell regeneration with a maximum of 18 % at a test concentration of 50 vol% of Bormia-Water©. All test concentrations ≥ 30 vol% were statistically significant ($p < 0.01$; Wilcoxon-Mann-Whitney-Test) from the initial tap water. The results clearly demonstrate that Bormia-Water© also promotes the cell regeneration process when taken regularly.

Cell culture with HL-60 cells

Description of cells and routine cultivation

The second set of experimentsprsented here were done with promyelocytes of the cell line HL-60 (ACC-3; ECACC 98070106; Leibniz Institute DSMZ - German Collection for Microorganisms and Cell Cultures, Braunschweig, Germany). The cells were routinely cultured in RPMI 1640 with 10% foetal calf serum and 0.5% gentamycin and incubated in an incubator at 37°C and in a humid atmosphere of 5% CO_2 and 95% air. The cells were routinely cultivated in suspension and subcultured regularly on every third day.

Under specific conditions such as cultivation in medium with 1.5 % dimethylsulfoxide for 5 to 7 days, HL-60 cells differentiate into so-called functional neutrophils which possess the properties of phagocytic and inflammatory cells (neutrophil granulocytes) in the blood. Besides their phagocytic activity in the blood against penetrated microbes, cells may also penetrate a pre-damaged tissue area and generate reactive superoxide anion radicals. This oxidative or respiratory burst may cause further cell damage and keep a chronic inflammatory process going.

HL-60 cells can also be grown in adherent state which allows to examine their energy metabolism and to evaluate the test substance's potential to act on the primary unspecific defense mechanism in the blood.

Potential for neutralizing endogenously formed radicals

HL-60 mass cultures were differentiated into functional neutrophils by treatment with dimethylsulfoxide. After several washing and centrifugation steps, the cells were stimulated to form superoxide anion radicals by addition of a phorbol ester. The generated radicals caused a cleavage of a tetrazolium dye WST-1 (Roche Diagnostics, Mannheim, Germany), which was also added to the reaction mixture. The stronger the cleavage of the dye and thus also its change in optical density, the more radicals were generated by the cells and present in the reaction mixture. If the radicals were inactivated by Bormia-Water©, the optical density changed less strongly. The optical density was continuously recorded on the Elisa reader (BioTek SLx808) as a differential

measurement ΔOD = 450 – 690 nm and evaluated after linear regression of the curves (10 to 30 min). The results were calculated as relative values in comparison to the tap water.

As shown in Fig. 3A, the flow of water through the Bormia Ananda 20 GS with gold/silver-coated, left and right spinning rhythmical spirals and with an additional crystal waterfall caused a pronounced and dose-dependent inactivation of superoxide anion radicals which were generated endogenously by functional neutrophils. At all concentrations of Bormia-Water© ≥ 20 vol% the increased inactivation was statistically significant ($p < 0.01$; Wilcoxon-Mann-Whitney test). The radical inactivation helps to reduce oxidative stress, but also might positively act on local chronic inflammatory processes which conincide with an enhanced level of radicals.

Energy metabolism of adherent HL-60 cells

HL-60 cells can also grow adherently after differentiation and can be examined for their activity without oxidative burst. For this series of experiments, adherently growing HL-60 cells were seeded at a density of 20,000 cells/well into 96-well culture plates and incubated for 24 h. Thereafter, reaction mixtures consisting of phosphate- buffered saline with calcium and magnesium and 5 mM glucose, the two test waters (Bormia-Water© and tap water) and the tetrazolium dye WST-1 were added. The optical density was continuously recorded as already described before. After linear regression of the curves (time interval 10 to 80 min). The results of Bormia-Water© were calculated as relative values in comparison to the initial tap water.

Fig. 3B demonstrates that Bormia-Water© was able to stimulate the energy metabolism of adherent HL-60 cells within 180 min after application when compared with the initial tap water without flow. However, the stimulation was only examined at test concentrations > 20 vol%, but then became very prominent.

Conclusions

In the experimental animal-free tests performed here with connective tissue cell cultures and differentiated HL-60 cells, Bormia-Water© has demonstrated its beneficial effects when compared with the initial tap water without flow. Therefore, from our experiments, the regular intake of Bormia-Water© can be highly recommended for the improvement and maintenance of well-being.

References
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