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Beneficial Effect of Sustainable Magnetic Treatment of Tap Water Investigations with Cultivated Intestinal Epithelial Cells

Peter C. Dartsch*

Dartsch Scientific GmbH, Institute for Cell Biological Test Systems, Auf der Vosshardt 25, D - 49419 Wagenfeld, Germany

***Corresponding author:** Peter C. Dartsch, Dartsch Scientific GmbH, Institute for Cell Biological Test Systems, Auf der Vosshardt 25, D - 49419 Wagenfeld, Germany

ABSTRACT

(Electro)magnetic fields have been used as a tap water treatment since more than 60 years to prevent water pipe walls from the unwanted deposition of calcium carbonate. In the meantime a lot of research has been done on the effects of magnetic or electromagnetic treatment of water. Most of them deal with physiochemical aspects and only a few report on biological effects on prokaryotes or cultured mammalian cells.

Intestinal health plays a key role in respect to systemic health and the intestinal epithelial barrier comes directly into contact with drinking water. The effect of magnetic tap water treatment on the integrity of the intestinal epithelial barrier was investigated by measurement of the transepithelial electrical resistance (TEER). Moreover, the sensitivity of the intestinal epithelial barrier against oxidative stress was investigated. Since the regeneration process of intestinal epithelial cells is essential for the reconstitution and maintenance of the barrier integrity after injury or traumatization *in vivo*, this aspect was also examined with water ± magnetic treatment. The mammalian cell line IPEC-J2 was used for the investigations. Local tap water (= initial source water) was obtained before and after magnetic treatment with water core magnets which show a very weak field strength with high magnetic gradients as a special characteristic.

TEER increased by 28.5 ± 6.8% after incubation with the tap water with magnetic treatment when compared to the initial tap water (mean value ± standard deviation; $p \leq 0.01$ by the two-tailed Wilcoxon-Mann-Whitney rank sum test). Measurement of TEER after oxidative stress showed no marked difference between both tap water samples ± magnetic treatment. In both experimental series there was a significant reduction in TEER by more than 90 % representing a leaky intestinal epithelial cell layer due to oxidative stress. Despite this fact, the number of cells in the initial tap water sample was largely decreased, whereas the cell density in the treated water samples was much higher. The magnetic treatment of the tap water improved the regeneration of the intestinal epithelial cells by 17.4 ± 7.2% (mean value ± standard deviation; $p \leq 0.05$ by the two-tailed Wilcoxon-Mann-Whitney rank sum test) when compared with the initial tap water.

The results of the study demonstrate that the water core magnets as used here are able to influence tap water and, subsequently, induce beneficial cellular effects on cultured intestinal epithelial cells. Thus, the tap water with magnetic treatment might be able to promote and maintain intestinal and systemic health *in vivo*.

Keywords

Magnetic water treatment
Tap water
Intestinal epithelial cells
IPEC-J2
Intestinal barrier
Transepithelial electrical resistance (TEER)
Cell regeneration
Oxidative stress
Reactive oxygen species

Research Article

INTRODUCTION

The intestinal barrier has several important immunological and non-immunological functions. The epithelial cell layer is one of the most important non-immunological components by providing a physical barrier between the contents of the intestinal lumen and the rest of the body, ensuring efficient absorption of essential nutrients from the intestinal lumen, and producing mucus and substances with regulatory properties. Intestinal hyperpermeability due to a reduced integrity of the juctional complexes between adjacent epithelial cells [1] has been shown to contribute to the pathogenesis of several gastrointestinal disorders, thus influencing not only intestinal health itself, but also systemic health [2-4]. Ingested materials and microbial pathogens which disturb normal cellular homeostasis in the intestine can induce oxidative stress and gastrointestinal injury by an excess of reactive oxygen species [5,6].

(Electro)magnetic fields have been used as a tap water treatment since more than 60 years to prevent water pipe walls from the unwanted deposition of calcium carbonate. In 1958, a first commercial application was presented in Belgium [7]. Over the past 40 years a lot of research has been done on the effects of magnetic or electromagnetic treatment of water. Most of them deal with physiochemical aspects [8-10] and only a few report on biological effects on prokaryotes [11,12].

Prompted by this background we investigated the effect of magnetic tap water treatment on the integrity of the intestinal epithelial barrier as well as their sensitivity against oxidative stress after application to cultured intestinal epithelial cells. Moreover, we also examined the regeneration process of intestinal epithelial cells to reconstitute and maintain the barrier integrity after injury or traumatization *in vivo.*

MATERIAL AND METHODS

Magnetic treatment of local tap water

Local tap water (= initial source water) was obtained before and after magnetic treatment with water core magnets (IPF GmbH, Austria). These magnets show a very weak field strength with high magnetic gradients as a special characteristic. The water core magnets have been used in recent studies and the magnetic field has been mapped in detail [13,14]. Only freshly produced water was used for the investigations and was added immediately after production to the culture medium in concentrations as specified in the experiments described below.

Cell culture

The investigations were conducted with IPEC-J2 cells (ACC-701; Leibniz Institut, DSMZ, Braunschweig, Germany). The cells were routinely grown in Dulbecco's Modification of Eagle's Medium (DMEM) supplemented with 10% growth mixture and 0.5% gentamycin and cultivated in an incubator at 37°C in an atmosphere of 5% CO_2 and 95% air at nearly 100% humidity. The cells were routinely cultivated as mass cultures and were regularly subcultured twice a week with fresh culture medium. For the experiments, cells were taken from 80-90% confluent mass cultures.

Examination of transepithelial electrical resistance (TEER)

IPEC-J2 cells were cultured for a total of 7 days on the surface of 0.4 µm porous membranes (Transwell plates, Corning, Sigma-Aldrich, Deisenhofen, Germany), which resulted in two separate compartments within the cell culture dish. The layer of cells covering the surface of the membrane (= apical compartment = equivalent to the intestinal lumen) represents a physical barrier to the lower compartment (= basolateral compartment = equivalent to the blood). Transepithelial electrical resistance (TEER) was measured by placing an electrode in the culture medium in the apical compartment and an electrode in the culture medium in the basolateral compartment [15]. TEER was measured directly with a portable voltmeter (Millicell ERS-2 voltmeter, Millipore/Merck, Darmstadt; Germany). Only intestinal epithelial cell layers with an electrical resistance of at least 2,000 Ω/cm^2 were used for the experiments, which represents an intact physical barrier with very good integrity [16-18]. TEER of the porous membrane without any epithelial cell barrier was measured with values between 160 and 180 Ω/cm^2.

Prior to TEER measurements, epithelial cell layers were cultured for two days with 25 vol% tap water ± magnetic treatment in the culture medium. Thereafter, TEER was measured again and the relative increase was calculated. Three independent experiments were conducted.

Examination of the integrity of the intestinal barrier after oxidative stress

Since an intact intestinal epithelial cell barrier with high integrity should be able to react less sensitive against oxidative stress than a barrier with "normal" integrity, 2 mmol/l hydrogen peroxide as a donor of reactive oxygen species was added to the culture medium of the apical compartment for 24 hours. In addition, 25 vol% tap water ± magnetic treatment was added to the apical compartment. TEER was examined again and the relative decrease was calculated. Finally, cell cultures were washed with phosphate-buffered saline, fixed with methanol, stained with Giemsa's azur eosin methylene blue solution (Merck, Darmstadt, Germany) and air-dried.

Examination of cell regeneration

The granulation phase, characterized by the occurrence of migration and proliferation of epithelial cells for closing a defect after injury, was simulated [19,20]. For this purpose, cells were seeded at a density of 100,000 cells/ml into the individual compartments of a silicone 4 well-culture insert (ibidi, Gräfelfing, Germany). The single compartments of the inserts are separated by a 500 µm thick silicone bar with an outer silicone frame of 700 µm. Due to the special adhesion area, a silicone insert adheres firmly to the bottom of a culture dish and forms a distinct cell-free area (artificial wound), which the cells can colonize by migration and proliferation.

Upon reaching cell confluency within 48 hours after cell seeding, the silicone frames were removed with tweezers to achieve a sharp edge of the cell-free area between the compartments. 25 vol% of tap water ± magnetic treatment was added to the intestinal epithelial cells in the dishes. Cells were allowed to migrate and proliferate for 18 hours. Finally, cell cultures were fixed with 100% methanol, stained with Giemsa's azur eosin methylene blue solution (Merck, Darmstadt, Germany) and air-dried. The colonized area was examined by micrographs and calculated by a specialized software with artificial intelligence from KML Vision, Graz, Austria (IKOSA AI software). Three independent experimental series (n=3) were conducted.

STATISTICAL ANALYSIS

Statistical analysis of data was done using the two-tailed Wilcoxon-Mann-Whitney rank sum test. Significance was assumed at $p \leq 0.05$ or $p \leq 0.01$ as indicated.

RESULTS

TEER increased by 28.5 ± 6.8% (mean value ± standard deviation) after incubation with the tap water with magnetic treatment when compared to the initial tap water. This represents a statistically significant improvement in epithelial barrier integrity by tap water with magnetic treatment ($p \leq 0.01$). Measurement of TEER after oxidative stress showed no marked difference between both, tap water samples ± magnetic treatment. In both experimental series there was a significant reduction in TEER by more than 90 % representing a leaky intestinal epithelial cell layer due to oxidative stress. However, as shown in Figure 1, there were morphological differences between the epithelial cell layers of both water samples. While the number of cells in the initial tap water sample was decreased so that you could even see the pores of the underlying semi-permeable membrane, the cell density in the treated water samples was much higher. Therefore, an injured intestinal barrier should be reconstituted much more quickly. The results on the intestinal barrier are expanded by the regeneration data. The magnetic treatment of the tap water improved the regeneration of the intestinal epithelial cells by 17.4 ± 7.2% (mean value ± standard deviation; ($p \leq 0.05$) when compared with the initial tap water. The residual cell-free space was still present in the case of the initial tap water after 18 hours, whereas the cell-free space was completely closed and filled with cells for the tap water with magnetic treatment (Figure 2).

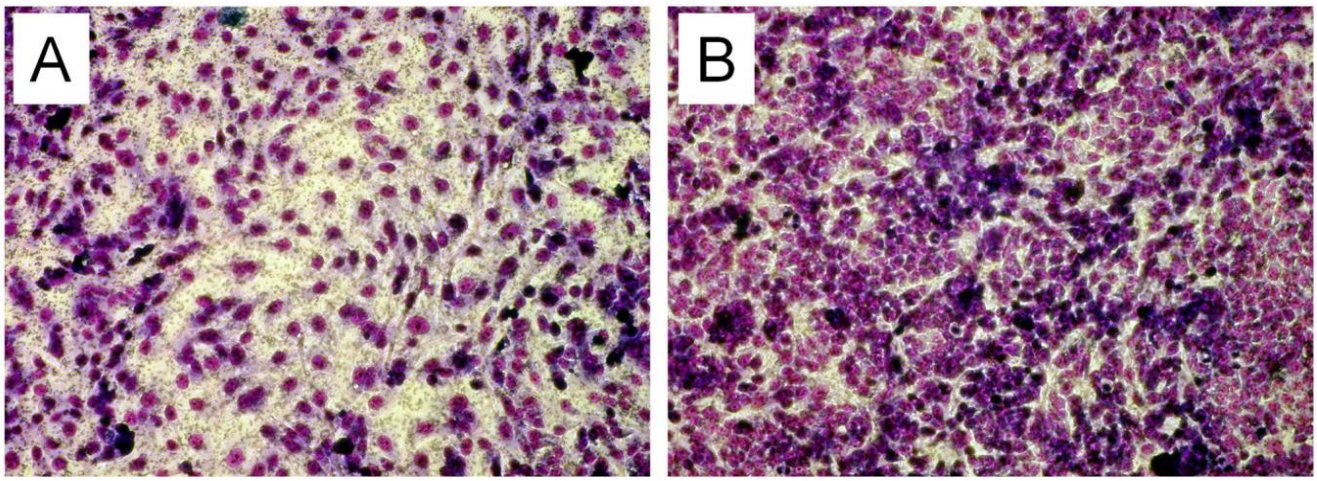

Figure 1: Representative micrographs of the fixed and stained cell cultures after exposure to 2 mmol/l hydrogen peroxide for 24 hours. (A) Cells in 25 vol% of initial tap water and (B) in 25 vol% of tap water with magnetic treatment. The difference in the remaining cell density is clearly visible. Olympus IX 50 inverted microscope at brightfield illumination with a planachromate 20x using an Olympus E-10 and 4 megapixel resolution.

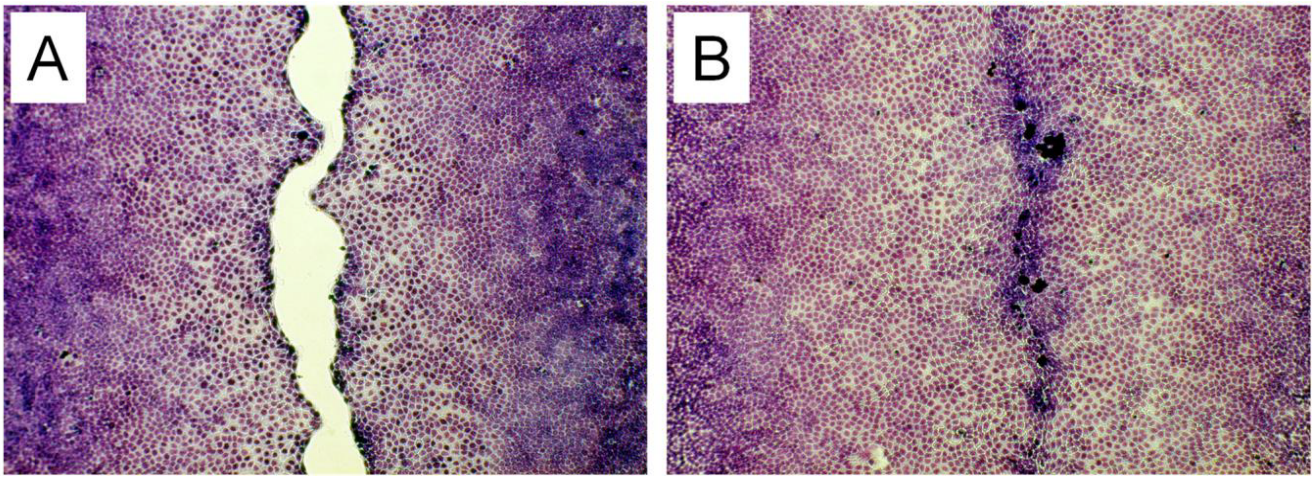

Figure 2: Representative micrographs of the fixed and stained cell cultures after 18 hours of regeneration. (A) Cells in 25 vol% of initial tap water and (B) in 25 vol% of tap water with magnetic treatment. The difference in the colonization of the cell-free space is clearly visible. Olympus IX 50 inverted microscope at brightfield illumination with a planachromate 10x using an Olympus E-10 and 4 megapixel resolution.

DISCUSSION

During the last years it has been shown in a number of studies that prokaryotes respond to the magnetic treatment of water [11,21,22]. Especially the publication of Liu et al. [12] gives a good overview on the current knowledge of the magnetic treatment on prokaryotes. They concluded "that magnetic field treatment can be a sustainable method for microbial community management". However, the mode of action of magnetic treatment on water still remains unclear and has led to several hypothesis as summarized by Mosin and Ignatov [23].

Although there are many studies which demonstrate the direct effect of (electro)magnetic fields on the growth of cultured mammalian cells [24,25], to our knowledge, the effect of magnetically treated water on cultured intestinal cells has not been investigated yet. Since the intestinal epithelial wall comes into direct contact after drinking of water, there is a high concentration of water which might influence physiological and biochemical reactions of the intestinal cells. Subsequently also systemic reactions of the organism can be influenced after water uptake. Therefore, the effect of tap water with and without magnetic treatment on cultured intestinal cells was investigated. Magnetic treatment was conducted by use of water core magnets which show a very weak field strength with high magnetic gradients as a special characteristic. The water core magnets have been already used in recent studies and the magnetic field has been mapped in detail [13,14].

The IPEC-J2 cell line was chosen for the examinations, because "the IPEC-J2 cell line is unique as it is derived from the small intestine and is neither transformed nor tumorigenic in nature. IPEC-J2 cells mimic the human physiology more closely than any other cell line of non-human origin" [26]. The cells were originally isolated in 1989 by Helen Berschneider at the University of North Carolina [27]. The advantage of the IPEC-J2 cell line as an in vitro model originates from its morphological and functional similarities with intestinal epithelial cells *in vivo* [28]. The epithelial cells of the intestinal barrier have a high turnover rate, because they are quite sensitive against alterations of their endogenous environmental conditions involving a deficiency of the epithelium and immune/inflammation mediating cells [6].

As demonstrated in this study, magnetic treatment of local tap water resulted in beneficial biological effects on the regeneration of cultured intestinal epithelial cells and on the integrity of the epithelial barrier when compared with untreated corresponding controls. Both cellular properties are essential to promote and maintain the integrity of the intestinal wall barrier. Especially regeneration plays an essential role after intestinal injury due to food intolerance, food additives and many other factors that might cause oxidative stress and, subsequently, a reduced intestinal permeability and function. Defects in intestinal barrier function play a pathogenic role in intestinal dysfunction and disease. As reviewed by Farhadi et al. [2], a role for reactive oxygen species "in gastrointestinal-related abnormalities has been established for several gastrointestinal disorders. These include ischemic injury of the gastrointestinal mucosa, inflammatory bowel disease, peptic ulcer disease…" and others. It is not surprising that

oxidative stress can damage the intestinal barrier, alter its functions and increase intestinal permeability. Therefore, in case the intestinal barrier is strenghtened by drinking water which has undergone magnetic treatment, it can resist more easily oxidative stress from exogenous factors and their unwanted injuries.

CONCLUSIONS

The investigations of the present study demonstrate very impressively that magnetic water treatment with special water core magnets causes significant beneficial cellular effects on the intestinal tract. Therefore, the use of water core magnets as used here can be highly recommended to promote intestinal and systemic health and well-being.

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