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Protective Effect of the QiBracelet® Against Oxidative Stress

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ABSTRACT

Environmental oxidative stress is caused by different artificial and natural sources (environmental stressors). These influences might increase the generation of reactive oxygen species which are known to cause unwanted adverse health effects. According to the manufacturer, the QiBracelet® contains a grid chip which forms a static field that stimulates water molecules to undergo a transition into the coherent state. Since our body consists of about 70-85 % of water, the coherent state of the water molecules should be able to influence the cells of our whole body in a positive manner. Thus, the cells might obtain the ability to compensate any environmental stressors causing oxidative stress much more efficient when compared with endogenous enzymatic systems.

The goal of this study was to investigate whether different cultured cell types were able to resist exogenous oxidative stress by addition of 2 mM hydrogen peroxide to the culture medium much more efficient in the presence of the QiBracelet® in comparison to unprotected control cells.

Without protection, connective tissue fibroblasts (L-929) were the most sensitive cell type, while lung cells (A-549) were the least sensitive; connective tissue fibroblasts showed a loss in viability of 24% and lung cells of 84%. The other cell types such as kidney cells (MDCK), liver cells (Hep G2) and intestinal epithelial cells (IPEC-J2) had a viability between the two values. Independent from cell type, the QiBracelet® demonstrated a protective effect against exogenous oxidative stress. The extent of this protection varied among cell types, with liver cells showing the highest level of protection at 47.3 ± 7.1 %, followed by connective tissue fibroblasts at 29.6 ± 5.6%, kidney cells at 27.1 ± 5.9%, intestinal epithelial cells at 18.0 ± 7.1%, and lung cells at only 3.9 ± 2.8%.

The results demonstrate that the use of the QiBracelet® was able to protect all tested cell types from the hydrogen peroxide-induced environmental oxidative stress. Therefore, the QiBracelet® might also promote and maintain a better well-being and attitude of life *in vivo.*

Keywords

Oxidative stress
Coherent water
MDCK
Hep G2
IPEC-J2
L-929
A-549
Cell viability
Cell culture

INTRODUCTION

The environment contributes significantly to human health and disease. Environmental oxidative stress is caused by different artificial and natural sources (environmental stressors) such as exposure to industrial pollution, traffic exhaust, solar and electromagnetic radiation, geopathic interference fields and many others. These influences are known to increase the generation of reactive oxygen species, which have adverse health effects and are believed to be a major contributor to public health [1-3]. Thus, reactive oxygen species, oxidative stress and the environment form a complex relationship.

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In addition, metabolic processes in the body that generate energy in the mitochondria always produce small amounts of reactive oxygen radicals, which play an important role in cellular signalling [4,5]. The resulting amount of radicals is normally inactivated by the body's own enzymes such as glutathione, superoxide dismutase, catalase and others. Environmental stressors and an unfavorable metabolic situation may result in an overall excess of reactive oxygen species causing not only damages at the molecular and cellular level, but also several human diseases and disorders [6-8].

According to quantum electrodynamics (QED), liquid water is a system of two phases in which one of the phases is in a coherent state with all molecules oscillating in the same phase, whereas the other is made up of uncorrelated molecules in a gas-like state [9]. The collison of such uncorrelated water molecules might interfere with cell communication and signalling [10-12]. In the case of coherent water, additional hydrogen bonds cause the water molecules to arrange themselves in a structure without any impact. Thus, all signals within the body should reach the cells in an unaffected way and influence them to resist environmental stressors in a more confident way [13-15].

According to the manufacturer, the QiBracelet®, as examined in the present investigation, contains a grid chip which forms a static field that stimulates water molecules to undergo a transition into the coherent state. The coherent state of the water molecules positively influences the cells of our whole body so that the cells compensate any environmental stressors which might cause unwanted oxidative stress. We used current bioassays with various cultured cell types to examine whether the use of the QiBracelet® might result in a protective effect against an overall oxidative stress.

MATERIAL AND METHODS

QiBracelet®

The QiBracelet® was kindly provided by Qi Blanco UG (haftungsbeschränkt), D-97711 Maßbach, Germany, for the duration of the experiments. According to the manufacturer, the device contains a grid chip which forms a static field that stimulates water molecules to undergo a transition into the coherent state. Since our body consists of about 70-85 % of water (depending on age), the coherent state of the water molecules should be able to influence the cells of our whole body in a positive manner. Thus, the cells might obtain the ability to compensate any environmental stressors causing unwanted oxidative stress much more efficient when compared with endogenous enzymatic systems.

Cell cultures

The investigations on oxidative stress presented here were performed with the following five different cell types:

(1) Madin-Darby Canine Kidney cells, MDCK (NBL-2), CCL-34, parent strain [16]. Cells were originally isolated in 1958 from normal kidney tissue from a normal, adult, female cocker spaniel [17]. Cells grow in a clustered manner.

(2) Hep G2 cells, ACC-180. Established from the tumor tissue of a 15-year-old boy in 1975 [18]. Cells grow adherently and epithelial-like as monolayers and in small aggregates.

(3) IPEC-J2 cells, ACC-701. Established in 1989 from normal intestinal epithelium cells isolated from the jejunum of a neonatal, unsuckled pig [19]. Epitheloid cells growing adherently as monolayer.

(4) A-549 cells, ACC-107, CCL-185. Established from an explanted lung tumor which was removed from a 58-year-old man in 1972; model for alveolar type II pulmonary epithelium [20]. Epithelial cells, growing adherently as monolayer.

(5) L-929 cells, ACC-2, also known as NCTC clone 929 Clone of strain L. Connective tissue fibroblasts established from the normal subcutaneous areolar and adipose tissue of a male C3H/An mouse [21]. Adherent fibroblasts growing as monolayer.

Cell strains no. (1) - (4) were routinely grown in a mixture of Dulbecco's Modification of Eagles Medium (DMEM; 1.0 g/L glucose) and Ham's F12 medium (1:1) supplemented with 10 % growth mixture and 1 % penicillin/streptomycin. Cell strain no. (5) was routinely grown in RPMI 1640 supplemented with 10 % growth mixture and 1 % penicillin/streptomycin. Cells

were cultivated in an incubator at 37 °C in an atmosphere of 5 % CO_2 and 95 % air at approximately 100 % humidity. Cells were routinely cultivated as mass cultures and were regularly subcultured twice a week.

For the experiments, cells were taken from 80-90% confluent mass cultures. All media and supplements were from PAN-Biotech, Aidenbach, Germany. Cell culture dishes and flasks were from Techno Plastic Products (TPP), Trasadingen, Switzerland.

Experimental design

In order to investigate the ability of intestinal epithelial cells to survive exogenous oxidative stress with and without the

positive impact of the QiBracelet®, the cells were seeded at a density of 100,000 cells/well into 96-well plates. After complete attachment and spreading of the cells within 48 hours, cells were exposed to hydrogen peroxide at concentrations ranging from 0.25 to 3 mM with and without the QiBracelet® by using two separate mini-incubators (Figure 1). Both

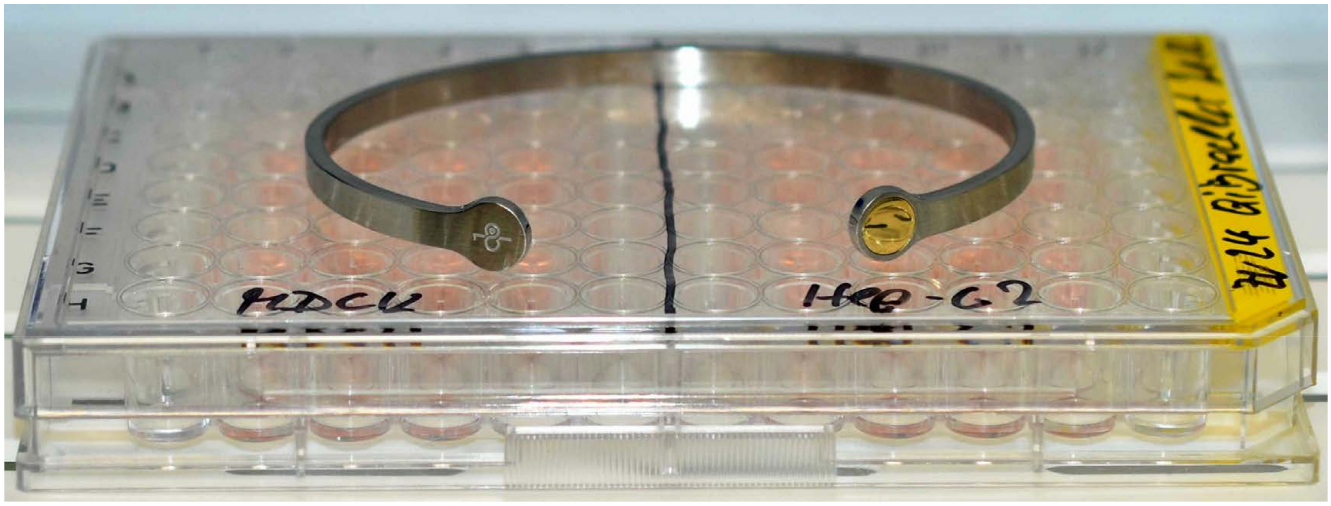

Figure 1: Arrangement of 96 well-plate which was placed within the mini-incubator during hydrogen peroxide-induced oxidative stress. The QiBracelet® was placed at the top of the 96 well-plate.

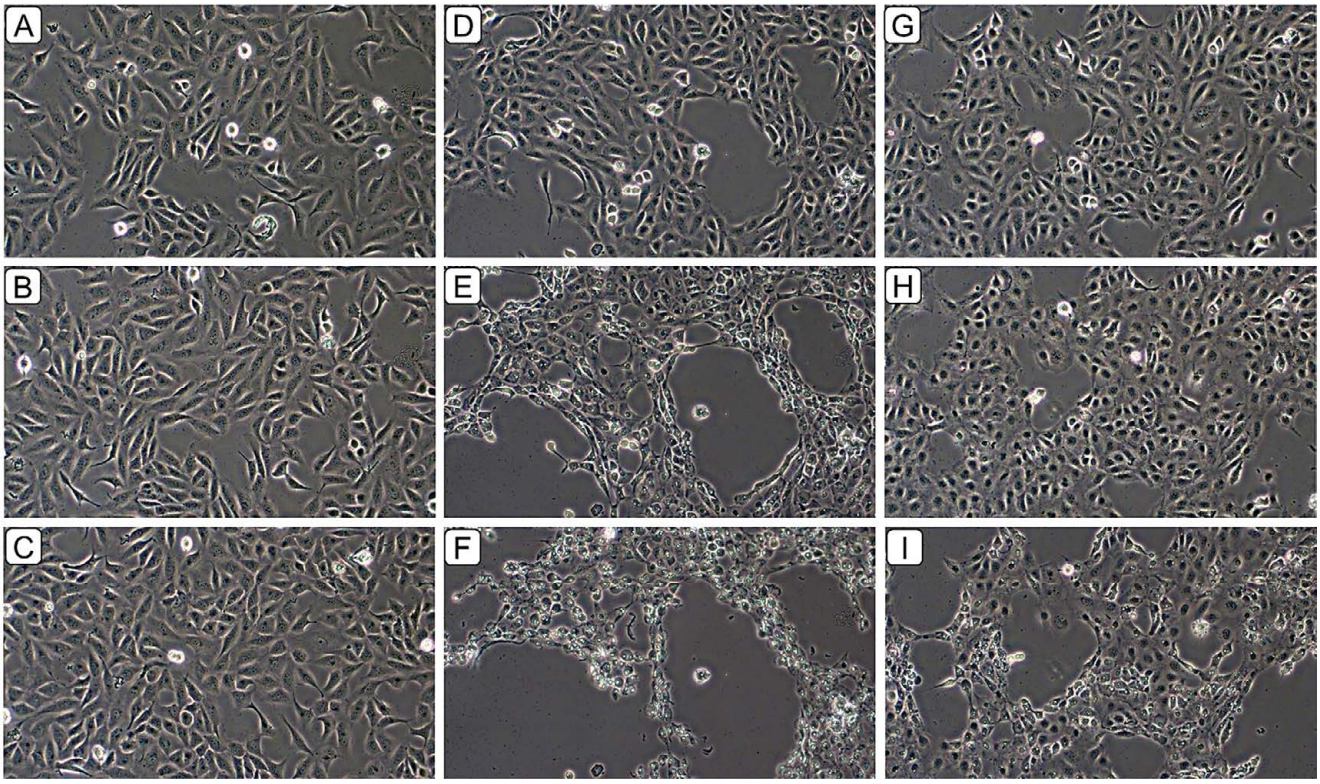

Figure 2: Micrographs demonstrating the effect of oxidative stress by hydrogen peroxide to cultured Hep G2 liver cells at different time points. Control cultures without hydrogen peroxide after 1 hour (A), 4 hours (B) and 8 hours (C). Unprotected cells with 2 mM hydrogen peroxide after 1 hour (D), 4 hours (E) and 8 hours (F). QiBracelet®-protected cells with 2 mM hydrogen peroxide after 1 hour (G), 4 hours (H) and 8 hours (I). Note that the protected cells show a significant lower rounding and detachment which represents cell traumatization and death. Olympus IX50 inverted microscope with 10x Planachromate at phase contrast. Micrographs were taken with an Olympus E20P at 5 megapixel resolution.

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mini-incubators were about 20 meters distant with several house wall between them. This guaranteed that there was no interference between the two different cell samples. After 24 hours the cells were washed with phosphate-buffered saline and fresh culture medium containing 10% of the water-soluble tetrazolium dye XTT (sodium-3′-[1-[(phenylamino)-carbony]-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene-sulfonic acid hydrate; Xenometrix, Allschwil, Switzerland) was added. Due to the activity of the mitochondrial enzymes in metabolically active cells, the initially slightly yellowish dye was cleaved and an orange color developed. The extent of the color change was proportional to cell vitality. This dye is widely used in a colorimetric assay for examination of cell viability and proliferation [22-24]. The optical density (= color change of the dye) was recorded at t = 0 and definite time points at ΔOD = 450-690 nm with an Elisa reader (BioTek ELx808 with software Gen 5 version 3.00) and finally calculated with Microsoft Excel. A total of three independent experimental series were performed over a period of 3 months with duplicate wells each.

STATISTICAL ANALYSIS

Statistical analysis was done with the non-parametric two-tailed Wilcoxon-Mann-Whitney test**.**

RESULTS

Hydrogen peroxide concentrations up to 1 mM did not have any negative impact on the cells, whereas a concentration of 3 mM resulted in almost complete cell death within 24 hours. As a result, a concentration of 2 mM hydrogen peroxide was chosen for further analysis (Figure 2). Without protection, connective tissue fibroblasts were the most sensitive cell type, while lung cells were the least sensitive. Thus, the sensitivity of different organ-specific cell type to exogenous oxidative stress induced by hydrogen peroxide varied within a wide range from 24% (L-929) to 84% (A-549) cell viability (Figure 3A). Independent from cell type, the QiBracelet® demonstrated a protective effect against exogenous oxidative stress (Figure 3B). The extent of this protection varied among cell types, with liver cells showing the highest level of protection at 47.3%, followed by connective tissue fibroblasts at 29.6%, kidney cells at 27.1%, intestinal epithelial cells at 18.0%, and lung cells at only 3.9% (Figure 3C).

DISCUSSION

Oxygen possesses two contradictory properties for biological systems, which are primarily beneficial effects such as the generation of large amounts of adenosine-5-triphosphate (ATP) through oxidative phosphorylation or oxygen radicals for

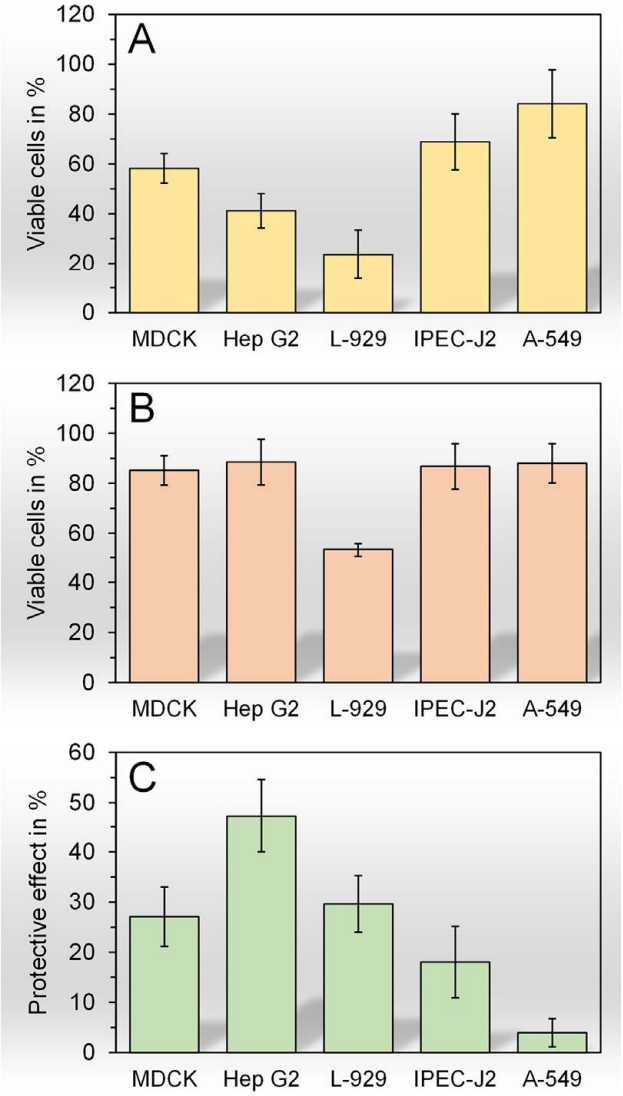

Figure 3: Presentation of the results on cell viability after 24 hours of oxidative stress without protection (A) and with protection by the QiBracelet® (B). The relative protective effect by the QiBracelet® is given in (C). Data represent mean values ± standard deviation of three independent experimental series.

cellular signalling, but on the other hand an excess of oxygen radicals can also cause potentially damaging effects [25-27]. In addition, reactive oxygen species are also generated as a response by a number of artificial and natural environmental stressors and can also induce oxidative stress [1-3].

Prompted by this background we investigated whether the QiBracelet® might be able to reduce the negative impact of oxidative stress coming from the cellular environment. Although the principles of quantum electrodynamics (QED) are not really accepted in conventional medicine as a method to influence the state of water, the present investigation has shown

that coherent water as generated by use of the QiBracelet® obviously had a positive impact on cells by increasing their resistance against environmental stressors.

Hydrogen peroxide was used in this study to simulate environmentally-induced oxidative stress to different cell types. The results of this study demonstrated that the different cell types had a diverging sensitivity against oxidative stress. This might be due to organ-specifity (skin and inner organs such as intestine, kidney, liver, lung) or as a result of the cell strain itself. In a variety of our previous toxicological studies, especially connective tissue fibroblasts always had a higher sensitivity in comparison to other cultured cell types. Thus, also in this present study connective tissue fibroblasts had the lowest viability of only 24% after oxidative stress, whereas the lung cells had the highest viability of 84%. The use of the QiBracelet® demonstrated that this product was able to protect all cell types of this study from the hydrogen peroxide-induced environmental oxidative stress. However, the degree of protection varied and was highest for liver cells and lowest for lung cells. This result showed that the protection from environmental oxidative stress by the QiBracelet® was an overall mechanism and not related to a single cell type.

As a conclusion, the QiBracelet® might also promote and maintain a better well-being and attitude of life *in vivo*. Further research is needed to understand the underlying mechanisms and potential applications of the QiBracelet® in protecting cells from oxidative stress.

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