The Influence of Torsion Field on Mouse Kidney Cells *In-vitro*

Lin Wang[1#], Zhongzhen Cai[1#], Yu Chen[1], Qian Feng[1], Mengmei Li[1], Yuming Chen[1], Jie Teng[1], Caixia Li[1], Peng Gao[1], Glen Rein[2], B. Qing Tang[1] and Xuemei Bai[1*]

[1]ENNOVA Institute of Life Science and Technology, ENN Group, Langfang 065001, China

[2]Quantum Biology Research Lab, PO Box 1402, Ridgway, CO, USA

[#]These authors contributed equally to this work

*Corresponding author: Xuemei Bai, ENNOVA Institute of Life Science and Technology, ENN Group, Langfang 065001, China

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Abstract

The objective of this study was to examine the biological effects of Poynting Vector based torsion fields on mammalian cells *in-vitro*. M-1 mouse kidney collecting duct cells were placed into the torsion fields, with the biochemical and genetic indicators measured at 24 and 48 hours and then compared with those of the control group. The results indicated that the total adenosine pool (TAP), glutathione (GSH) levels, the genes of *Hsf1* and *Hsp32* were significantly increased, while the reactive oxygen species (ROS) and mitochondrial copies were significantly decreased. Many other factors remained unchanged including mitochondrial membrane potential (MMP) levels, superoxide dismutase (SOD) levels, telomerase, telomere length, cellular growth, cellular viability, and the genes of *Bcl-2, P16, P21,* and *Sirt1*. Based on the results, we conclude that mitochondria, which act as a cell signal transmission platform and can engage in intracellular signal processing, can be influenced by torsion fields. In addition, cell metabolic capacity, defense and stress resistance are significantly enhanced after short-term exposure to torsion fields.

Abbreviations: TAP: Total Adenosine Pool; GSH: Glutathione; MMP: Mitochondrial Membrane Potential; SOD: Superoxide Dismutase; EM: Electromagnetic; ROS: Reactive Oxygen Species; FBS: Fetal Bovine Serum; RPV: Rotating Poynting Vector; SD: Standard Deviation; OD: Optical Density

Introduction

The bioelectromagnetics community has repeatedly demonstrated that Electromagnetic (EM) force fields affect biological systems by modulating a variety of biochemical pathways [1]. The Society for Bioacoustics has also clearly demonstrated that acoustic sound energy has biological effects at the clinical and cellular levels with a focus on ultrasound [2]. In addition to sound, light at various wavelengths has a variety of biological effects [3]. In addition to these classical forms of energy, there are non-classical energy fields which do not obey the classical equations of motion described by Maxwell [4]. One example is the longitudinal wave (also called potential fields), which are modified EM fields [5]. Longitudinal waves exist in the human body [6] and exogenously generated longitudinal waves have biological effects [7]. Another type of non-classical, non-EM field is called a spinor field in the

western publications and the torsion field in the Russian literature. Technically, a torsion field is a type of EM field with circular polarization under torsion stress. These fields are described in the physics literature in terms of their spin angular momentum [8] and their polarization effects on the vacuum [9].

The electric charge and gravitational fields of all rotating objects generates a torsion field, which have some unexpected properties [10]. Similar to the gravitational field, the torsion field can't be shielded by any natural material. Unlike EM fields which propagate along a transverse or longitudinal axis, torsion fields propagate with clockwise (right handed) or counterclockwise (left handed) spin. This is similar to other non-classical fields which propagate in a helicoid [11] or spiral manner [12, 13]. Torsion fields propagate through all medium but unlike EM fields, their amplitude does

not fall off as $1/r^2$ [14], thereby allowing for non-local effects [10]. Theory further predicts that torsion fields do not possess energy or mass, but only information. In this sense torsion fields are similar to Qi as described below and longitudinal waves [15], as these energies can be considered information field. Mitochondria are vital organelles for an individual organism, mainly because of their role in energy metabolism and biosynthesis. However, a growing number of studies have shown that mitochondria, performing various signal transduction functions, act as a platform to initiate cellular signaling. Furthermore, mitochondria act as sensors and effectors in multiple biological processes, including ATP and reactive oxygen species (ROS) generation, related to cellular signaling pathways [16]. In these processes, the mitochondrial outer membrane serves as the primary signaling system which regulates cellular signaling through mitochondrial dynamics and metabolites.

Most importantly, defects in mitochondria that regulate homeostasis and control cell signaling may be the basis of age-related pathology [17] and many other diseases. As a cell signaling platform, mitochondria are constantly engaged in intracellular signal processing and transmission. This raises the question how mitochondria react and respond to signals outside the cell? Here we consider the possibility that such signals could be transferred via endogenous non-classical energy fields. To test this hypothesis, we investigated the effect of torsion fields on the energy metabolism of mouse kidney collecting duct cells (M-1). Reactive oxygen species, senescence related indexes such as telomerase, telomere length, TERT, *Bcl-2, P16, P21* and other senescence related genes were also examined, to explore the possible influence of torsion fields on cell senescence.

Materials and Methods

Cell Culture

The M-1 mouse kidney collecting duct cell line was obtained from National Infrastructure of Cell Line Resource (Beijing, China, http://www.cellresource.cn) as a visceral cell which grows rapidly. The M-1 cells were seeded at a density of $5\sim6\times10^5$ cells/plate into 10cm plates and cultured as monolayers using DMEM/F12 1:1 medium supplemented with 10% fetal bovine serum (FBS) in a humidified 5% CO_2 incubator at 37°C. The medium was changed 2–3 times per week. Stock cells were grown in 10cm culture dishes and split at a sub-cultivation ratio of 1:5.

Torsion Field Generator

Torsion fields were generated using a RPV type emitter. The basic principle of Rotating Poynting Vector (RPV) generator, originally designed by Akimov [9], is based on the quantum spin-spin interaction of orthogonal magnetic H and electric E fields generating a spiraling S-vector (Figure 1). We built our own RPV generator according to Akimov's specifications, except a stronger neodymium magnet was placed at the bottom of the generator. The generator consists of a copper cone, with an underside diameter

of 76mm and a height of 48.8mm (in according with the golden ratio). Between the copper cone and the copper coil (made of 23gauge solid copper wire) is a 2mm thick plastic cone, made by a 3D printer. When an electric signal is applied to the copper cone and the copper coil (see the two input wires in (Figure 1), a 10^6V/m electric field is generated, and whose direction is vertical to the inclined plane of the copper cone. The electric signal was generated by a high voltage AC positive pulse generator with a voltage of 2000-3000 Volts and a frequency of 13kHz. The electric current is not relevant here because in this generator only the voltage signal is used to generate the electric field between the copper cone and the copper coil. In this case, the component of the electric field will be orthogonal to the vertical magnetic field, a prerequisite to generate a torsion field. Therefore, a right-handed spiral RPV field will be generated around the plastic cone.
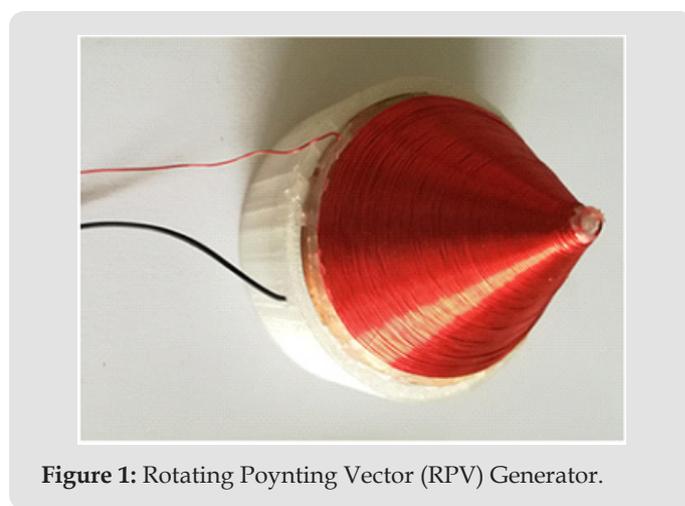
Figure 1: Rotating Poynting Vector (RPV) Generator.

Application of Torsion Field to Cells

The M-1 cells were seeded at a density of $5\sim6\times10^5$ cells/plate into 10cm plates. After cultivating in the same incubator for 24h, triplicate plates with cells in the logarithmic growth phase were placed in another incubator containing the right-hand torsion field. Plates were stacked on top of each other on a shelf above the RPV generator, with the bottom plate about 4cm away from the tip of the RPV generator. For the control group, the triplicate plates were left in the original incubator for normal cultivation. Other than the torsion field treatment, these two incubators were set at exactly the same conditions, while being located in two separate rooms about 10 meters away. Cells for both the control and the treatment groups were incubated for 48h, with subsamples collected at 24h and 48h respectively during incubation.

Biochemical Analysis

Measurement of ATP, ADP, and AMP Levels: A new HPLC analysis was employed to study ATP, ADP, and AMP in M-1cells [18]. An aliquot of 1ml cell samples was centrifuged at 10,000g for 5min to collect cells, followed by the addition of 100μl PBS and 40μl deionized water. After that, 360μl perchloric acid (6%)

was added to remove the protein by keeping samples on ice for an additional 10min. The cell extract was centrifuged at 10,000g at 4°C for 5min, with 300μl supernatant neutralized with 40μl of 2 M K_2CO_3, and filtered through a 0.45μm filter. A 10μl neutralized cell extract was used for determination of ATP, ADP, and AMP, which was carried out using HPLC with mobile phase (0.1M KH_2PO_4 buffer at pH 6.25) and 5% methanol (v/v) set at a rate of 0.6ml/min and a detection wavelength of 254nm. ATP, ADP and AMP quantization was calculated by computing peak areas, and injecting standard solutions of nucleotides with known concentrations. The total adenylate pool (TAP) was calculated by the following formula: [TAP]=[ATP]+[ADP]+[AMP]. Finally, TAP levels in cells were normalized to the total cell protein.

Measurement of ROS Levels: ROS levels were quantified in accordance with the protocol of the reactive oxygen species assay kit (Product No.: S0033; Beyotime, China). An aliquot of 0.5ml cell samples was incubated with DCFH-DA solution (2μM) at 37°C for 40min in the dark. Then samples were washed two times with PBS for measurement. The fluorescence intensity was recorded at 485nm (excitation) and 535nm (emission) using a micro plate reader (Tecan, Switzerland). Total ROS levels in cells were normalized to the total cell protein.

Measurement of Mitochondrial Membrane Potential: MMP levels were quantified in accordance with the protocol of the Mitochondrial Membrane Potential Assay Kit (Product No.: C2006; Beyotime, China). To study the MMP levels in cells, 0.5ml aliquots of the cell samples were incubated with a JC-1 probe at 37°C for 20 min in the dark. After the incubation, cells were washed twice with ice-cold JC-1 buffer solution. The JC-1 probe entering the mitochondrial matrix will aggregate and emit at 590nm (red) upon excitation at 525nm, whereas the monomeric JC-1 remaining in the cytoplasm emits at 530nm (green) upon excitation at 490nm. The fluorescence intensity was recorded using a microplate reader (Tecan, Switzerland). The value of MMP calculated as the ratio of red to green.

Measurement of Protein: Cell quantities were evaluated as the protein concentration using a Protein Assay Kit (Product No.:P0006C; Beyotime, China) with bovine serum albumin as the standard (0~1.5mg/ml). An aliquot of 0.5ml cell samples was centrifuged at 3000g for 5min to collect cells, which was then disrupted with Triton (0.1%) for 30min. After cell disruption, samples were centrifuged at 11000g for 5min to remove cell residues. The supernatant was used for optical density measurements at a wavelength of 595nm by using a microplate reader (Tecan, Switzerland).

Measurement of Cell Viability

Cell viability was evaluated by using the Cell Counting Kit-8 (CCK-8) (Product No.: C0038x; Beyotime, China). Briefly, an aliquot of 0.5mL cell samples was centrifuged at 3000g for 5min to collect cells, which were then re-suspended in 100μl culture medium and seeded in 96-well plates. Finally, 10μl CCK-8 was added to the wells and the plates were incubated for an additional 3h. The optical density (OD) was measured at a wavelength of 450nm using a microplate reader (Tecan, Switzerland).

Measurement of Antioxidant Levels: Antioxidant levels in cells were evaluated by the enzyme activity of SOD and the levels of GSH, using an SOD Assay Kit with WST-8 (Product No.: S0101; Beyotime, China) and a total Glutathione Assay Kit (Product No.: S0052; Beyotime, China) following the manufacturer's protocols. The absorbance of SOD and GSH was assessed at 450nm and 412nm respectively using a microplate reader and the final SOD activity and GSH levels in cells were normalized to the total cell protein.

Mitochondrial DNA Quantification and Gene Expression Levels by q-PCR

Mitochondrial DNA Quantification: Total DNA was extracted from M-1 cells using the MiniBEST Universal Genomic DNA Extraction Kit (TaKaRa, 9765) according to the manufacturer's protocols. The number of mtDNA copies was quantified by real-time PCR using TB Green® Premix Ex Taq™ II (TaKaRa, RR820) and a quantitative real time-PCR system (ABI, QuantStudio3). Primers specific for mitochondrion DNA and nuclear gene B2M are listed in (Table 1). The relative abundance of mtDNA copies was obtained by normalization to B2M using the $2^{-\Delta\Delta Ct}$ method.

Table 1: Primers used in this study.

Primers	Sequence	Accession
β-actin-Mouse-F1	AGATCAAGATCATTGCTCCTCCT	NM_007393.5
β-actin-Mouse-R1	ACGCAGCTCAGTAACAGTCC	
Sirt1-Mouse-F1	GATACGGAGAGGCCCGAATG	AY377984.1
Sirt1-Mouse-R1	AGTTCCCAATGCTGGTGGAG	
p21-Mouse-F1	CCCGAGAACGGTGGAACTTT	AB017818.1
p21-Mouse-R1	GAGTGCAAGACAGCGACAAG	
Hsf1-Mouse-F1	CATGAGCCTGCCTGACCTG	NM_001331152.1
Hsf1-Mouse-R1	CACCAGCTGCTTTCCTGAGT	

p16-Mouse-F1	TTCTTGGTGAAGTTCGTGCG	BC058190.1
p16-Mouse-R1	CGTGAACGTTGCCCATCATC	
Hsp32-Mouse-F1	GCCTCCAGAGTTTCCGCATA	NM_010442.2
Hsp32-Mouse-R1	AGGAAGCCATCACCAGCTTAAA	
Bcl2-Mouse-F1	GGGCTACGAGTGGGATGC	Reference [19]
Bcl2-Mouse-R1	CCACCGAACTCAAAGAAGG	
mB2M-F1	ATGGGAAGCCGAACATACTG	NC_000068.7
mB2M-R1	CAGTCTCAGTGGGGGTGAAT	
mMito-F1	CTAGAAACCCCGAAACCAAA	NC_005089.1
mMito-R1	CCAGCTATCACCAAGCTCGT	

Gene Expression Levels: Total RNA was extracted from M-1 cells using the MiniBEST Universal RNA Extraction Kit (TaKaRa, 9767) according to the manufacturers' protocols. RNA was then reverse transcribed into cDNA using Reverse Transcriptase M-MLV (TaKaRa, 2641A). Gene expression was quantified by real-time PCR using TB Green® Premix Ex Taq™ II (TaKaRa, RR820) and a quantitative real time-PCR system (ABI, QuantStudio3). The primers specific for *Hsf1, Hsp32, Bcl-2, P16, P21, Sirt1* and **β**-actin are listed in (Table 1). The relative abundance of each target was obtained by normalization to **β**-actin using the $2^{-\triangle\triangle Ct}$ method.

Statistical Analysis Values of different measurements were normalized to a respective mean control value from the untreated samples and expressed as percent control. To evaluate treatment effects, the results of treatments at 24h and 48h were combined as a whole (divided into treated and control groups) for analysis. All data were expressed as mean **±** standard deviation (SD). Comparisons between the control and treated groups were done with the Student's t-test using the GraphPad InStat software for analysis, where P<0.05 was considered statistically significant.

Compared with the control group, there were many changes in the cells that were treated by the torsion field, with a significant increase in TAP production (Figure 2a), a significant reduction in ROS levels (Figure 2b) and mitochondrial copies (Figure 2d). However, the MMP remained unchanged (Figure 2c). This result indicated that the torsion field significantly increased the metabolic capacity while reducing the harmful oxygen-containing free radicals. The observed decrease in mitochondrial copies, however, may be due to environmental influences on the replication of mitochondrial DNA. The influence of torsion field on the cellular self-protection mechanisms was also investigated. As shown in (Figure 3), the SOD showed no significant changes (a), but the GSH levels increased significantly in treated cells (b), indicating an enhanced antioxidant capacity of the cells.
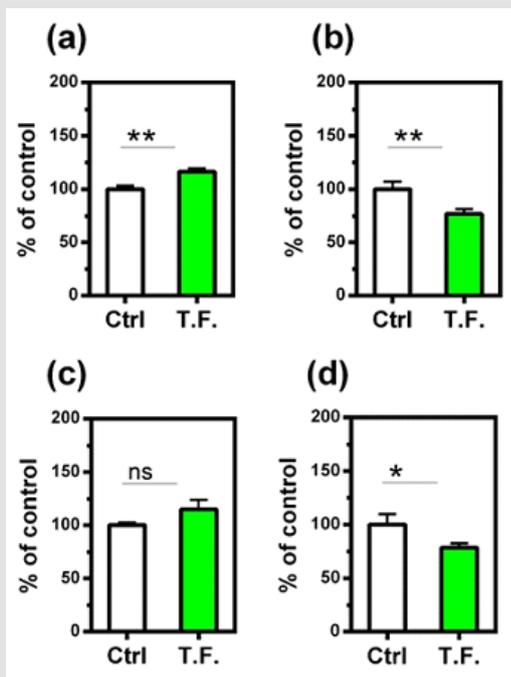
Figure 2: Effect of torsion field treatment on mitochondrial functions in M-1 cells

• TAP (total adenylate pool, TAP=ATP+ADP+AMP, ATP: adenosine triphosphate; ADP: adenosine diphosphate; AMP: adenosine monophosphate);

• ROS;

• MMP;

• Mitochondrial copies.

* P < 0.05; ** P < 0.01, when compared with the untreated control using Student's t-test.
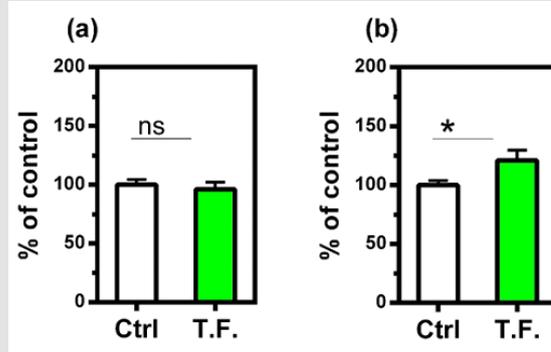
Figure 3: Effect of torsion field treatment on antioxidant related items in M-1 cells

• SOD;

• GSH. *P < 0.05;

**P < 0.01, when compared with the untreated control using Student's t-test.
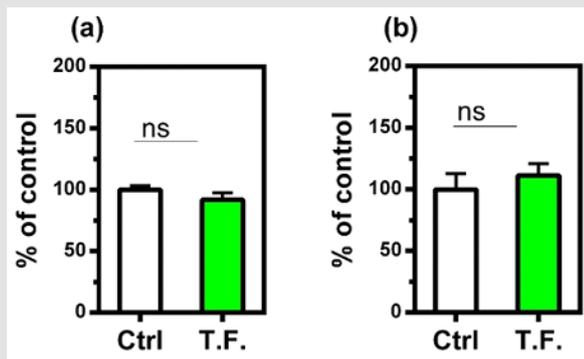
Figure 4: Effect of torsion field treatment on telomere related items in M-1 cells

- telomerase;
- telomere length.

*P < 0.05; **P < 0.01, when compared with the untreated control using Student's t-test.

The effect of the torsion field on the telomeres of the cells was also investigated. Neither the telomerase nor telomere length in the cells showed a significant difference (Figure 4). It can be seen in (Figure 5), changes in cell growth and activity were not identified. After treatment with a torsion field, the gene expression in cells also varied, with a significant enhancement in *Hsf1* and *Hsp32* (Figure 5a & Figure 5b), but no changes for other genes (*Bcl-2, P16, P21* and *Sirt1*) (Figure 5c-5f). The increase in these two genes indicates that the ability of cells to resist stress and maintain homeostasis is enhanced. This in turn promotes the self-protection of cells.
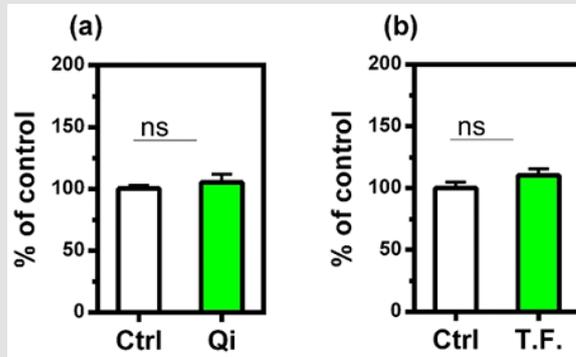
Figure 5: Effect of torsion field treatment on cellular growth and cellular viability in M-1 cells

- cellular growth;
- cellular visibility.

*P < 0.05; **P < 0.01, when compared with the untreated control using Student's t-test.
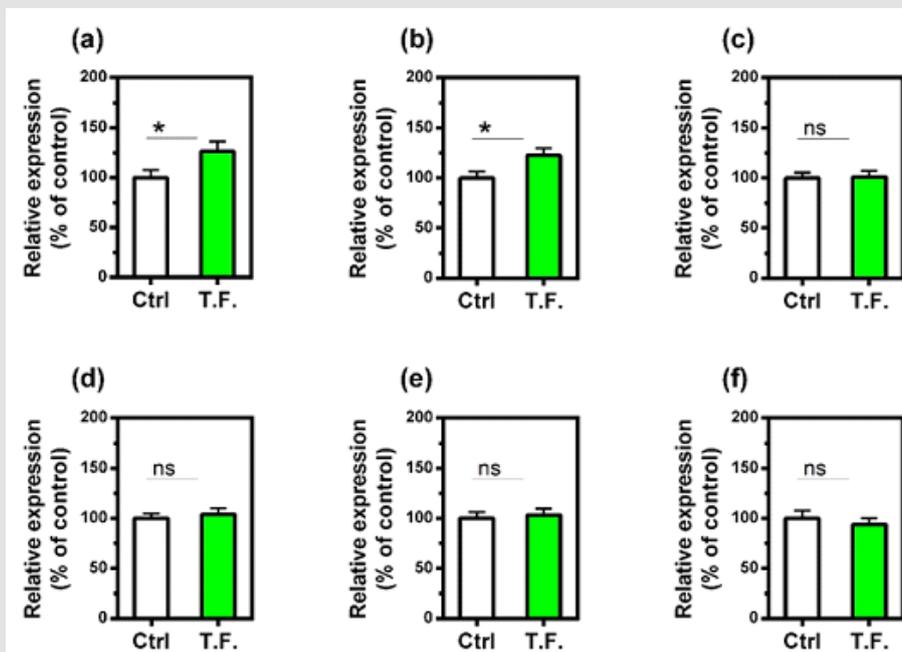
Figure 6: Effect of torsion field treatment on gene expression in M-1 cells

- *Hsf1;*
- *Hsp32;*
- *Bcl-2;*
- *P16;*
- *P21;*
- *Sirt1.*

Discussion

Although transverse EM fields are well established to effect biological systems. Circularly polarized light and torsion fields are examples of spinor fields. Spin waves, although studied in the physics literature, are not often used in biological experiments. Theory predicts that when torsion fields interact with matter, they only affect the spin state of matter. Russian scientists have a long history of research with torsion fields because they have a strong theoretical [9] and experimental [20] frame work and a way to generate torsion fields in the lab. In fact, there are several types of torsion fields and several types of generators to broadcast torsion fields. Of the different types of torsion field generators which have been scenically studied, generators based on the Poynting vector have been extensively studied [20, 21] and were used in the present study. A recent study by our group demonstrated some temperature anomalies with such torsion fields. However, the biological effects of these torsion fields have not been studied outside of Russia. Although there are numerous claims about the health benefits of right-handed torsion fields and the detrimental effects of left-handed fields in the Russian literature. In general, left-handed fields accelerate life processes like germination [22] and right-handed fields reverse life process such as ripe peanut germination [23] and refolding of denatured proteins [24]. Unfortunately, there few studies have been published in peer-reviewed western journals.

Even fewer studies are published regarding the biological effects of torsion fields. However, Lupichev demonstrated a non-local intercellular communication between cells in separate petri dishes [25]. Effects of torsion fields on the germination of Arabidopsis thaliana seeds, however, have been reported [22]. In this study, both right-handed and left-handed torsion fields stimulated seed germination by approximately 30%. A similar experiment with similar results was conducted by Szabo using Spruce seeds treated with an unconventional torsion field generator [26]. The present study is the first to demonstrate biological effects of Poynting Vector based torsion fields on mammalian cells *in-vitro*. The results indicate that ATP production and GSH levels were significantly increased, oxygen-containing free radicals were significantly decreased whereas SOD levels were unchanged in cultured mouse kidney cells treated with a home-made torsion field generator. This indicates that the antioxidant capacity of the cells was enhanced to mainly scavenge peroxide-based free radicals [27]. This conclusion is supported by the additional finding that torsion fields reduce ROS levels.

Nonetheless, these effects are target specific molecules, since telomerase and telomere length were not altered and are process specific, since cell growth was unaffected. Nonetheless, it is presently unclear whether torsion fields primarily interact with the plasma membrane or with intracellular proteins and organelles. However, the results of the present study indicate that torsion fields can interact directly with mitochondria. Mitochondria are well known to play an important role in the homeostasis regulation of cells. Thus, our results show that the mitochondrial mtDNA copy numbers are decreased significantly following the torsion field treatment, indicating the restricted replication of mitochondrial DNA. We also observed an increase in mitochondrial stability and cellular indicators related to longevity and aging (apoptosis) following torsion field treatment. However, torsion field's effects on mitochondria are specific since the mitochondrial membrane potential (MMP) is unaffected. Nonetheless, mitochondria appear to be act as an antennae and are able to receive signals from torsion field which initiate the regulation of oxidative phosphorylation and superoxide free radical metabolism. At the nuclear level, our results indicate that torsion fields only affect specific genes. Thus, we observed a significant increase in *Hsf1* and *Hsp32* gene expression.

This indicates that at least in kidney cells, an enhanced ability of the cells to resist stress and maintain a steady state. The effects of torsion fields are specific since senescence genes (*Bcl-2, P16, P21, Sirt1*) showed no response. Although torsion fields are different than classical EM fields, as discussed in the introduction, their biological effects can be similar. Here, we demonstrated one type of torsion field stimulates TAP, and others have shown that EM fields, of a different frequency, can also increase ATP synthesis in erythrocytes [28]. In contrast to the anti-oxidant effects reported here, most EM fields have pro-oxidative capacity *in-vitro* [29]. We conclude that mitochondria, which act as a cell signal transmission plat form and can engage in intracellular signal processing, can be influenced by torsion fields. In addition, cell metabolic capacity, defense and stress resistance are significantly enhanced after short-term exposure to torsion fields. While mitochondrial DNA replication is limited, mitochondria stability is maintained and supported by indicators of longevity and aging (apoptosis), thus further confirming the huge role of mitochondrial homeostasis regulation.

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