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Effect of Electric Fields on Normal Fibroblast (BJ) Cells

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Abstract: ***Background: Electric fields (EFs)has been reported to affect living tissues.Effects of EFsas anticancer have beenrecently developed. Designing a therapy that specifically recognizeand kills cancer cells without affecting normal cells, has been a recentobjective in theranostics. Thus, this study aimed to evaluate the effect of EFs on normal fibroblast cells (BJ cells). Method: Cells were incubated for 2, 4, and 6 days with frequency of 50 KHz, 100 KHz, 150 KHz, 200 KHz, 300 KHz. Parameters measured were cells number and viability. Cells number and viability assesment was performed with MTS. Results: Number of BJ cells increased each day, compared to 0 day. Number of cells in each day showed no significant difference among treatment of EFs. There was no significant difference of cells viability among treatments each day, and among treatments-0 days, that indicate EFs showed no toxicity toward BJ cells. Conclusion: The result of present study showed there was no significant difference among treatment of EFs in normal fibroblast cells. These results indicate EFs did not affect proliferation in normal cell.***

Keywords: Electric fields, fibroblast

1. Introduction

Electric fields (EFs)has been reported to affect living tissues. Their activity ranged from stimulating excitable tissues such as nerve, muscle or heart in frequency-dependent manner [Pol, 1995; Palti, 1966], through stimulating bone growth and accelerating fracture healing [Besset, 1985], to using it for diathermy and radiofrequency tumour ablation [Chou, 1995]. Intermediate-frequency EFs (>10 KHz to MHz) were mostly considered as having no biological effect [Elson, 1995] and, hence, medical application, though several non-thermal cellular effects have been observed [Zimmerman et al, 1981; Holzapfel et al, 1982; Pawlowski et al, 1993].

Recently, in vitro and in vivo studies on effects of alternating EFsas anticancer, have been documented [Kirson et al, 2004; Kirson et al, 2007; Zimmerman et al, 2012; Barbault et al, 2009]. These studies showed that anticancer effects were achieved at specific (for the cancer cell type) modulation frequencies and demonstrated proliferative inhibition and mitotic spindle disruption following exposure to alternating electric fields [Kirson et al, 2007; Zimmerman et al 2013].Designing a therapy that specifically recognizeand kills cancer cells without affecting normal cells, has been a recentobjective in theranostics [Kievit and Zhang, 2011]. Thus, it is important to observe the effect of EFs on normal cells. In this study, we evaluated effect of EFs on normal fibroblast cells (BJ).

2. Materials And Methods

Cell Culture

BJ cells [ATCC®CRL-2522] were provided by Biomolecular and Biomedical Research Center, Aretha Medika Utama. Cells were grown in α-Minimum Essential Medium Eagle(α-MEM) [Biowest L0475], 10% Fetal Bovine Serum (FBS) [Biowest S181H], 1% Pennicilin Streptomycin [Biowest L0022], and maintainedat 37°C in humidified atmosphere and 5% CO_2 until the cells were 80-90% confluence. Growth medium was removed and washed with phosphate buffer saline (PBS) [Gibco 14200075]. Cells were then added with trypsin-EDTA [Biowest L0931-500], incubated at 37°C for 3 min. Tripsynization was stopped by adding growth medium in equal volume. Cells were suspended and replaced into tube, centrifuged at 500 x*g*for 4 min. Supernatant was removed and pellet were resuspendedwith 4-5 mL growth medium. Cell suspension was aliquoted into T-flask containing growth medium with density of8000 cell/cm^2.Medium was replaced every two days. Cells were incubated at 37°C, 5% CO_2.

Treatments

Cells were washed with 1 mL 1X PBS twice.Cell were then added with 1 mL trypsin EDTA, incubated for 3 min at37°C, 5% CO_2.Cells were removed to tube containing 5 mL culture medium, and centrifuged at 500 g for 5 min.Supernatant was removed, cells were resuspended with 1 mL culture medium.Cells were plated into 96 wellswith 5000 cells eachwellin 100 µl medium. . Cells were incubated for 2, 4, and 6 days at 37°C, 5% $CO_2$with frequency of50 KHz, 100 KHz, 150 KHz, 200 KHz, 300 KHz.Cells number and viability were measured with 20µL (3-(4,5-dimethylthiazol-2-yl)-5-(3 carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) [Promega, Madison, WI, USA],and incubated at 37°C for 3 hours.Absorbance was measured usingmicroplate reader (MultiSkan-Go)at 490 nm wavelength.

3. Results

Cell number and viability were evaluated by MTS assay. MTS assay is a colorimetric method for determining the number of viable cells in proliferation or cytotoxicity assays (Malich *et al.*, 1997, Widowati et al., 2013). Absorbance was measured using microplate reader at 490 nm wavelength. Cells number of BJ cells can be seen in Table 1.

Table 1: Effect various incubation periode and various electric frequency toward cells numebr in BJ cells

Treatments		Cells		
	0 day (Untreated)	2 days	4 days	6 days
Control	8,421±397	11,237±549	13,161±1,755	12,650±1,246
50 KHz		11,155±330	12,128±600	11,989±794
100 KHz		11,294±712	11,835±721	11,805±670
150 KHz		11,025±1,280	12,082±948	11,959±1,493
200 KHz		11,596±564	11,209±602	10,947±417
300 KHz		11,949±768	11,376±975	10,937±2,196

Data is presented as mean±standard deviation. Statistical analysis was performed with ANOVA and showed there was no significant difference of cells number among EFs each day.

As shown in Table 1, number of cells in each day showed no significant difference among treatment of EFs.

The BJ cell viability assay was the preliminary study to test cytotoxic of EFstoward BJ cell. Viability was indicated by the conversion of yellow tetrazolium salt to form a purple formazan product (Percentage of viable cells was determined by comparing cells viability value of treatments to the control. BJ cells viability can be seen in Table 2.

Table 2: Viability of BJcellstreated with different EFs in 2, 4, 6 days, and its comparison to 0 day

Treatments	Viability (%)					
	2 days	4 days	6 days	2 days over 0 day	4 days over 0 day	6 days over 0 day
Control	100.00±0.00	100.00±0.00	100.00±0.00	133.64±6.53	156.53±20.87	150.45±14.82
50 KHz	99.37±2.80	93.42±12.73	95.33±8.48	132.66±3.92	144.24 ±7.13	142.59 ±9.45
100 KHz	100.77±8.86	91.38±13.83	94.40±13.99	134.32±8.47	140.76 ±8.57	140.40±7.97
150 KHz	98.13±10.35	93.61±17.47	95.37±15.42	131.12±15.23	143.69 ±11.28	142.23 ±17.76
200 KHz	103.38±6.70	86.32±11.53	87.03±6.17	137.91±6.71	133.31 ±7.16	130.19±4.96
300 KHz	106.57±8.80	87.70±13.08	85.90±12.09	142.11±9.13	135.29 ±11.60	130.07 ±26.12

Data is presented as mean±standard deviation. Statistical analysis was performed with ANOVA, showed there was no significant difference of cells viability among treatments each day, and among treatments-0 days.

As shown in Table 2, there was no significant difference of cells viability among treatments each day, and among treatments-0 days. These results indicate EFs did not affect viability of normal fibroblast cells and considered safe to be further used.

Table 3: Inhibition of BJ cells treated with different EFs in 2, 4, 6 days, and its comparison to 0 day

Treatments	Inhibition					
	2 days	4 days	6 days	2 days over 0 day	4 days over 0 day	6 days over 0 day
Control	0.00±0.00	0.00±0.00	0.00±0.00	-33.64±6.53	-56.53±20.87	-50.45±14.82
50 KHz	0.63±2.80	6.58±12.73	4.67±8.48	-32.66±3.92	-44.24±7.13	-42.59±9.45
100 KHz	-0.77±8.86	8.62±13.83	5.60±13.99	-34.32±8.47	-40.76±8.57	-40.40±7.97
150 KHz	1.87±10.35	6.39±17.47	4.63±15.42	-31.12±15.23	-43.69±11.28	-42.23±17.76
200 KHz	-3.38±6.70	13.68±11.53	12.97±6.17	-37.91±6.71	-33.31±7.16	-30.19±4.96
300 KHz	-6.57±8.80	12.30±13.08	14.10±12.09	-42.11±9.13	-35.29±11.60	-30.07±26.12

Data is presented as mean±standard deviation. Statistical analysis was performed with ANOVA and Tukey post hoc that showed there was no significant difference of inhibition among treatments each day, and among EFs each day.

As shown in Table 3, there was no significant difference of cell inhibition among treatments each day, and among treatments-0 days. These results indicate EFs did not inhibit proliferation of normal fibroblast cells.

4. Discussion

EFs affect intracellular behaviour and interaction among cells. Biological electric plays role in extracelullar and intracellular ion exhange in maintaining cell physiology (McCaig et al, 2005).EFs have antimitotic effects through microtubular spindel breakdown during mitosis, that causes dielectrophoresis dislocation in intracellular molecules and organelles during cytokinesis, resulting in cell membrane damage and death (Eilon et al, 2004; Eilon et al, 2007).The result of present study showed there was no significant difference among treatment of EFsin normal fibroblast cells.Referring to Polk (1995), alternating EF of intermediate frequencies (10 kHz to 1 MHz) were considered not to have any meaningful nonthermal biological effects.Low frequencies of alternating EFs(under 1 kHz) stimulate excitable tissues through membrane depolarization (Polk, 1995). As the frequency of the EF increases above 1 kHz, the stimulatory effect diminishes. At very high frequencies (*i.e.*, above many MHz), a completely different biological effect is observed. At these frequencies,tissue heating becomes dominant due to dielectric losses. This effect becomes more intense as frequency, field intensity, or tissue dissipation factor increases (Elson, 1995).

In this study, EFs did not affect proliferation in normal fibroblast cell. For our best knowledge, there are only few

studies that report on EFs effect on normalfibroblast cell. Erickson and Nuccitelli (1984) found that fibroblast cells responded to EFs in three ways: (a) the cells migratedtowards the cathodal end of the field (b) the cells oriented their long axes perpendicular to the field lines(c) the cells elongated under theinfluence of field strengths of 400 mV/mm and greater.Anincrease in calcium translocation and the number of insulinreceptors were also documented in human fibroblast in response to an EFs [Bourguignon, 1989], that might be due to opening of voltage-sensitivecalcium channels as the primary event by EFs. Morever, EFs have been reported to generate morphological changes in normal human skin fibroblast with exposure of 0.1 and 0.4 V/mm. Human skin fibroblast react to an external EFsby significant reorientation of their cell bodies, in a perpendicular manner to the EFs[Methot et al, 2001]. However, underlying mechanism of such event remains unclear.

In cancer cells, recent studies showed alternating EFs with intermediate frequency (100-500 KHz) inhibits apoptosis via anti-microtubuler in cancer cell growth in breast cancer, lung cancer, and gioma cell line(Eilon et al, 2004; Eilon et al, 2007).However, molecular mechanism of EFs remains unclear. Possible mechanism occurred is via signal transduction (Koga et al, 2012).EFs activate PI3K (*phosphatidyl- Inositol 3-OH-kinase*)-*Aktpathway*in wound healing process, and p38 MAPK (*Mitogen Activated Protein Kinase*) *pathway*(Koga et al, 2012; Fukuda et al, 2013). Interestingly, EFs induces p53, enhances transcriptional function of p53, and G2 arrestin cell cycle in human epithelium cells through activation of p21 by p53 that play role as inhibitory protein of Cdk2 (inhibition of synthesis) and Cdc2 (inhibition of mitosis), resulting in G2 *arrest* (Fukuda et al, 2013).

5. Conclusion

The result of present study showed there was no significant difference among treatment of EFs in normal fibroblast cells. These results indicate EFs did not affect proliferation and untoxic in normal cell.

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