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ORIGINAL ARTICLE

TERM

Effects of Estrogen on Intracellular Calcium-Related T-Lymphocyte Function

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Regulation of immune cell function is an important in the field of hormone-related tissue engineering and regenerative medicine. In this sense, hormonal regulation of immune cell function is a critical issue to be solved. It has been known that ovarian sex hormone play an important roles in immune function, however, little has been known whether estrogen affects T-lymphocyte function. Human Jurkat T cells were treated with estradiol (E_2) at concentrations of 0, 10, 100, 1000 ng/mL, and calcium response was evaluated. Intracellular calcium concentrations after Fura-2 acetoxymethyl ester treatment show an increasing trend at higher E_2 concentrations although these alterations did not reach a statistical significance. The expression of calcium channel-related gene CACNA1C did not show any significant changes according to the concentration of E_2. Taken together, estrogen has an implication as a possible hormonal regulator of intracellular calcium release in human Jurkat T cells via non-genomic pathway. Further studies are necessary to investigate the combined effects of sex hormones and cytokines in both T- and B-lymphocytes.
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Key Words: Estrogen; T-lymphocyte; Calcium channel

INTRODUCTION

Regulation of immune cell function is an important in the field of hormone-related tissue engineering and regenerative medicine. For example, female aging is characterized by a decline in estrogen levels and by alterations of immune function. In this sense, hormonal regulation of immune cell function is a critical issue to be solved, especially in women who go through dynamic and cyclic changes in sex hormone levels [1]. It has been known that female sex hormone secreted from the ovaries play an important roles in immune function [2,3]. Relevance of female hormone is strongly suggested since autoimmune diseases are as high as 3−10 times of normal population [4].

The existence of estrogen receptor (ER)-α and ER-β on T-lymphocytes and B-lymphocytes was confirmed by polymerase chain reaction [5]. They act as intracellular transcription factors, and the expression level of ER-α and ER-β is not affected by hormonal status or other excitatory stimulus. When murine T cells are treated with female hormones, intracellular calcium concentration rises, which is considered to mediated by transmembrane receptors through non-transcriptional pathways [6]. This kind of calcium release shows a low amplitude and a short duration, and is deemed to be non-influential on the transcriptional and cellular function. However, recent studies revealed that the non-transcriptional pathways can affect the function of macrophages and their transcription [7].

Calcium channels are important for the function of T-lymphocytes. The calcium channel CaV1.2 is produced from the CACNA1C (calcium channel, voltage-dependent, L type, alpha 1C subunit) gene [8]. These channels, which transport calcium ions into cells, play a key role in a cell's ability to generate and transmit electrical signals. Calcium ions are involved in many different cellular functions, including cell-to-cell communication.

To date, little is known in regard to whether the estrogen affect the T-lymphocyte function, especially in terms of intracellular calcium release. In this investigation, human Jurkat T cells were treated with various concentrations of estradiol and post-treatment intracellular calcium changes and CACNA1C gene expression were evaluated.

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MATERIALS AND METHODS

Maintenance of T lymphocyte cell line and estradiol treatment

Human Jurkat T cells (CRL-2063, USA) were purchased from American Tissue Culture Collection. T cells were maintained as suspension in RPMI 1640 media (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, HyClone, South Logan, USA), 50 U/mL penicillin and 50 μg/mL streptomycin (Invitrogen). Cultured T cells were treated with estradiol (E_2, Sigma-Aldrich, St. Louis, MO, USA) at the concentrations of 0, 1, 10, 100, 1000 ng/mL for 48 hrs.

Activation of T-lymphocytes

The grown human Jurkat T cell was activated by CD3 antibody. Briefly, 5 μg/mL solution of anti-CD3 monoclonal antibody in PBS was plated and incubated at 4°C overnight. And then, the CD3 containing solution was removed and rinsed twice with PBS. For the preparation of T cells, 1×10^6 cells were suspended in RPMI 1640 media and cells were added to precoated dish and incubated for 2 days.

Intracellular calcium concentration measurement

The E_2-treated T cells were collected and re-suspended the cells with DPBS (Ca^{++}, Mg^{++} free). The cells were treated with 3 μM of Fura-2 acetoxymethyl ester (Fura-2 AM, Molecular Probes, Carlsbad, CA, USA), incubated for 30 min at 37°C, and washed with PBS by centrifugation. These cells were re-suspended in PBS and re-plated on confocal dish. The fluorescence was observed under fluorescence microscope (Nikon, Japan). To measure the fluorescence, 200 μL of suspended T cells was plated in 96-well plate and observed using Epoch microplate spectrophotometer (BioTek Instruments, Winooski, VT, USA) at spectrum 480 nm.

Quantitative reverse transcription-polymerase chain reaction

Total RNAs were extracted using Trizol (Invitrogen). Complementary DNAs were synthesized from 1 ug of total RNAs and Accute premix (Bioneer, Daejeon, Korea). And then, primer set for CACNA1C (forward: TCTTTCACCCCAATGCCTAC, reverse: TGCTG GAACATCTGCTATGC) were added and amplified under following condition, incubation for 10 min at

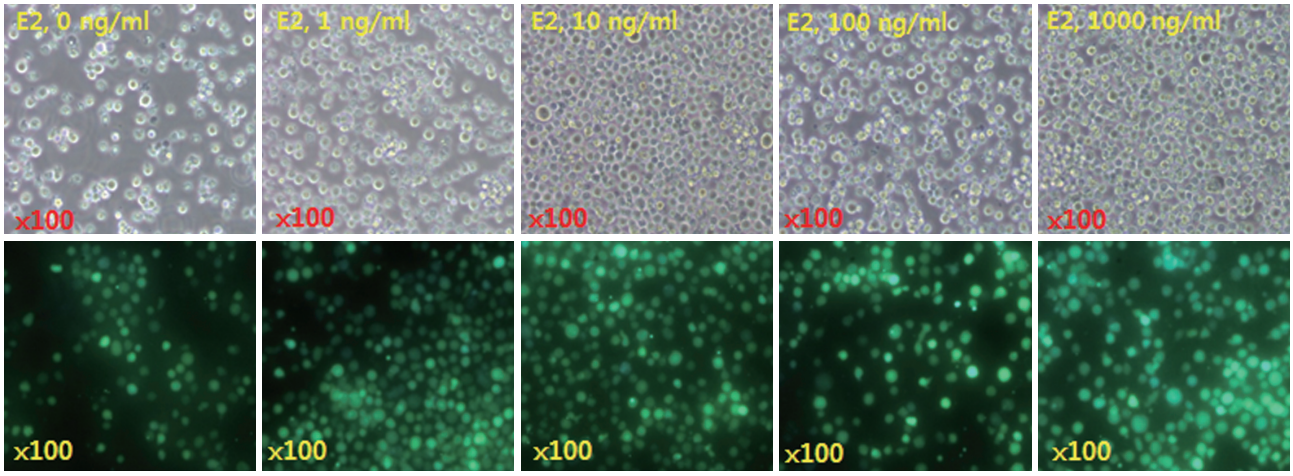

Figure 1. Intracellular calcium distribution in Jurkat T cells after treated with various concentrations of estradiol (E_2).

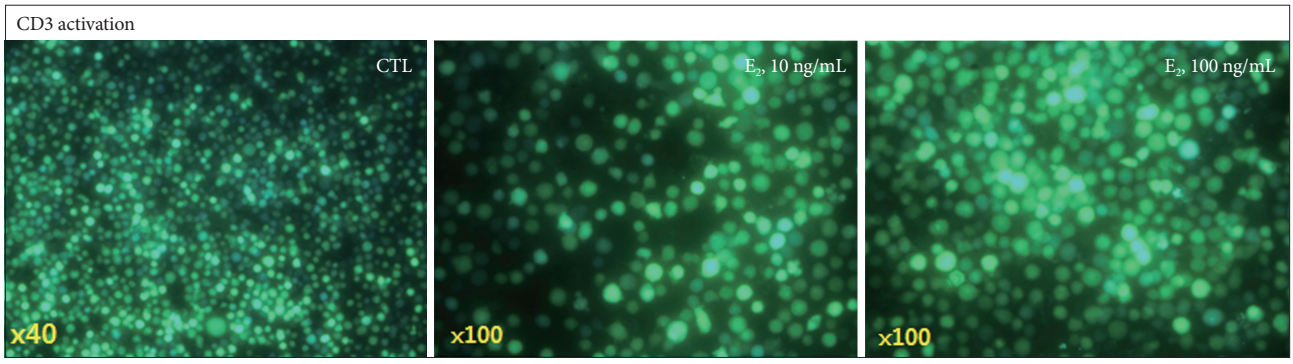

Figure 2. Fluorescent image was captured after Fura-2 AM staining. Post-CD3 activation intracellular calcium distribution in Jurkat T cells after treated with various concentrations of estradiol (E_2).

95°C, denaturation for 15 sec at 95°C, annealing for 40 sec at 58°C and 20 sec for 72°C for extension. All the reactions were performed as triplicate and expression of Ct was calculated based on the GAPDH expression.

Statistical analysis

The intensity and number of cells were calculated semi-quantitatively and compared between four different E_2 concentration groups and control group. Student t-test, χ^2 test and ANOVA with *post hoc* test were used for parametric and non-parametric comparison of groups and $p<0.05$ was considered as a statistical significance.

RESULTS

Intracellular calcium concentrations after Fura-2 AM treatment

When human Jurkat T cells were treated with Fura-2 AM in various E_2 concentration groups, intracellular calcium distribution was successfully observed. Although E_2-treated groups showed an increasing tendency compared to control and the intensity seemed to be proportional to the concentration of E_2 treated, this trend failed to reach a statistical significance (Fig. 1).

Post-CD3 activation intracellular calcium concentrations after Fura-2 AM treatment

When pre-treated for CD3 activation, human Jurkat T cells treated with Fura-2 AM showed an increasing trend in 100 ng/mL E_2 group compared to control (Fig. 2). The E_2 at 1, 10 ng/mL groups did not show any significant differences when compared to control group.

Quantification of intracellular calcium concentration using Fura-2

When intracellular calcium concentration was measured using Fura-2, E_2 at 100 ng/mL and 1000 ng/mL groups showed an increasing tendency compared to control (Fig. 3). E_2 1 ng/mL and E_2 10 ng/mL groups did not show any significant differences when compared to control group.

Expression of calcium channel-related gene CACNA1C

To semi-quantitatively evaluate the expression of calcium channel-related genes, the expression of CACNA1C was analyzed. When human Jurkat T cells were treated with various concentrations of E_2, the expression level of CACNA1C did not show any significant differences compared to the control group. (Fig. 4).

DISCUSSION

Despite considerable evidence that estrogen concentration affects immune function in women, there are very few reports on the exact proof that estrogen regulates intracellular calcium release. There are conflicting results regarding the increment of autoimmune disease prevalence in women who are treated with estrogen [9]. Little is known on the alteration of each component of immune cell system when female hormones levels are changed.

In our study, when intracellular calcium concentration was measured using Fura-2, the expression of calcium channel-related genes showed an increasing tendency when human Jurkat T cells were treated with E_2 at 1000 ng/mL, although parametric or non-parametric statistical analysis failed to reach a significance (Fig. 3). It may be inferred that the non-transcriptional influence of female hormones on the intracellular calcium concentrations of human Jurkat T cells is not strong and that this action mediated via calcium channel is minimal.

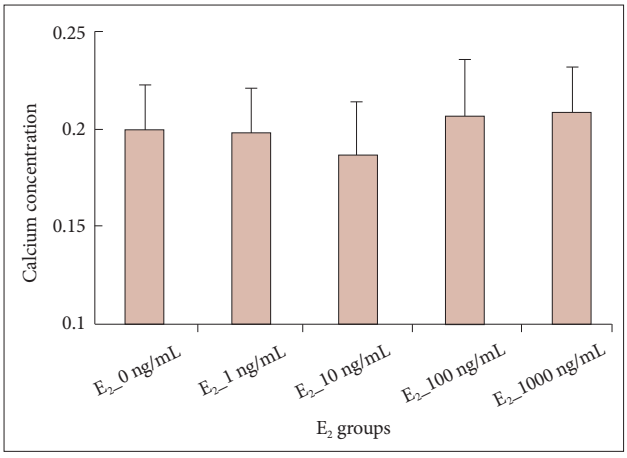

Figure 3. Intracellular calcium concentrations in various estradiol (E_2) concentration groups.

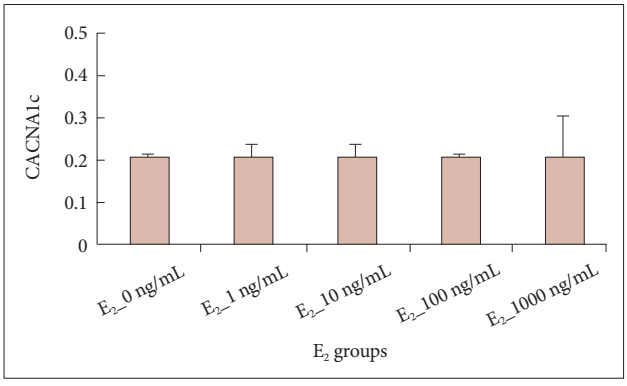

Figure 4. The expression of calcium channel-related gene CACNA1C on human Jurkat T cells after treated with estradiol (E_2) at 0, 1, 10, 100, and 1000 ng/mL of concentrations.

One previous study suggested a regulatory role of bisphenol A in the CACNA1C expression in human oral epithelial cells [10]. This aromatic compound has a similar structure to estrogens which also possess a benzene ring. Speculatively, estradiol was expected to regulate the expression of CACNA1C gene downward or upward, however, we did not observe any significant changes of its expression (Fig. 4).

Intriguingly, despite the changes shown in Figure 1, the expression of calcium channel-related gene, CACNA1C, was not up- or down-regulated according to various E_2 concentrations (Fig. 4). Probable alterations shown from Figures 1, 2, and 3 are divergent from the relative unchangeable calcium channel gene expression. This observation suggests the role of estrogen on T-lymphocytes via non-transcriptional pathways.

Some previous reports the role of estrogen inducing apoptosis of human Jurkat T cells [11-14]. This critical contradictory results from ours may have been culminated from the differences of hormone concentrations used. In reproductive age women, serum estradiol levels lie in the range of 30−300 pg/mL [1], and these levels decline further in post-menopausal years [15]. Aforementioned studies employed the concentrations of up to 100 μg/mL which corresponds to 100-fold to 100000-fold of the concentrations used in our study.

This study has some limitations. Our data indicated that the E_2 concentration of 1000 ng/mL showed an increasing tendency in terms of intracellular calcium concentration and calcium channel-related gene expression, when compared to control (Figs. 3 and 4). However, this concentration is a supra-physiological level considering the 30−120 pg/mL is used as a reference value when interpreting serum E_2 level in reproductive age women. The E_2 level may reach over 200−300 pg/mL immediately before ovulation and may reach up to 1000−2000 pg/mL, therefore, the concentration that showed a difference in this investigation seems still as high as can be deemed as supra-physiological.

In conclusion, human Jurkat T cell function may be regulated by E_2 treatment *in vitro* in terms of intracellular calcium release independent of calcium channel-related gene expression. Further studies are necessary to investigate the combined effects of estrogen and other sex hormones and stage-specific cytokines.

Acknowledgements

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Conflicts of Interest

The authors have no financial conflicts of interest.

Ethical Statement

The study was approved by the Institutional Review Board of the Seoul National University Hospital.

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