ISSN 2229-5518

Tamoxifen: A Novel Approach for the Treatment of Estrogen Receptor Negative Cancers

Eun Be Kim, Judith Jacobson, Donna Leonardi

Abstract-Tamoxifen is a selective estrogen receptor modulator that is used to treat estrogen receptor positive cancers, specifically breast cancer. Tamoxifen antagonizes the estrogen receptor and thus impedes estrogen induced growth of cancer cells. However, recent studies have shown that tamoxifen also inhibits protein kinase C (PKC), a protein that activates the PKC/ Akt/ PI3k pathway. Disrupting this pathway results in the inhibition of the downstream target of the PKC/ Akt/ PI3k pathway, mTOR, a protein that promotes cell survival and growth. The aim of this project was to determine the inhibitory activity of tamoxifen on an estrogen receptor negative (ER-) cell line, specifically a Burkitt's lymphoma cell line, Ramos. Enzyme immunosorbent assays were used to measure activities of PKC and mTOR. The MTS assay was used to measure Ramos cell viability after tamoxifen treatment (1-25μM). Results showed that Ramos cell viability, PKC activity, and mTOR activity each decreased with statistical significance demonstrating a dose-response relationship ($p < 0.05$) after tamoxifen treatment (1-25μM). Tamoxifen may therefore have potential as a treatment for not just ER positive cancers, but cancers that have a constitutively activated PKC/PI3k/Akt pathway.

Index Terms- Burkitt's Lymphoma, Estrogen Receptor, Inhibitor, Mammalian target of rapamycin, Protein Kinase C, Ramos, Tamoxifen

1 INTRODUCTION

BURKITT'S lymphoma (BL) is a highly aggressive non-Hodgkin B-cell lymphoma that affects B-lymphocytes. It is endemic among children in Sub-Saharan Africa, although it affects adults as well, and is associated with HIV [11]. Although Burkitt's lymphoma is one of the fastest growing malignancies in the pediatric population [2], with an appropriate treatment regimen, it is highly curable in first world countries. However, it still remains fatal in third world countries, and this disproportionality is caused by the unstable African governments that cannot afford to support the costly regimens, which include multiple therapeutics. Nor can the health care systems in these countries support the palliative care necessary due to the serious adverse effects of these regimens which include such drugs as methotrexate, which is delivered in high-dosage. Thus, it is crucial to develop novel BL treatments that are both effective and affordable and decrease the incidence of adverse reactions.

Researchers and pharmaceutical companies continuously attempt to discover alternative treatments to address the needs of specific demographics of the population. This is accomplished by the work of specialists in cellular biology and oncology who frequently seek ways to disrupt the intracellular signaling pathways and the proteins involved in the progression of the disease and this is particularly true in BL.

Researchers also investigate "off label use" of FDA approved drugs which can be efficacious, safe, and are cost effective.

• *Eun Be Kim is currently a high-school senior at Bergen County Academies in Hackensack, New Jersey. E-mail: eunbkim@bergen.og*

• *Dr. Judith Jacobson is Associate Professor of Clinical Epidemiology at the Columbia Mailman School of Public Health, NY, NY. Email: jsj4@columbia.edu*

• *Donna Leonardi, MS is currently the Director of the Bergen County Academies Laboratory of Cell Biology. E-mail: donleo@bergen.org*

This study investigated the use of tamoxifen, a drug used in the treatment of estrogen receptor (ER) positive cancers as a novel contribution to the treatment regimen for Burkitt's lymphoma using the ER negative BL cell line, Ramos, as the *in vitro* model system.

Protein kinase C (PKC) is a serine/ threonine kinase involved in signal transduction affecting proliferation, survival, and tumorigenesis [7]. PKC activates phosphatidylinositol 3-kinase (PI3K), which is a major enzyme in the signal transduction to downstream pathways that promote growth, proliferation, survival, differentiation, and metabolism in many cells [7]. When activated, PKC generates phosphatidylinositol-3,4,5-triphosphate, which activates Akt [14]. Akt then activates its downstream targets, such as the mammalian target of rapamycin (mTOR) [15]. The PI3K/Akt/mTOR pathway plays a crucial role in the development and progression of many solid tumors and hematologic malignancies [7]. Although PI3K plays a key role in cell growth signaling in many lymphoid malignancies, its role has not been fully elucidated [9].

Tamoxifen (TAM) is the best-known of a family of agents called selective estrogen receptor modulators (SERMs), used to treat breast cancers that have the receptors for the hormone, estrogen. Tamoxifen prevents endogenous estrogen from binding to the estrogen receptors within cancer cells, thereby slowing their estrogen induced growth [1]. However, recent studies have shown that tamoxifen also inhibits PKC signaling in estrogen receptor independent cancer cell lines, thereby suppressing the

PI3k/Akt pathway [5]. The Ramos (RA 1) Burkitt's lymphoma cell line has a constitutively activated PKC/PI3k/Akt pathway [12]. It was hypothesized that treating Ramos cells with tamoxifen would inhibit the PKC/PI3k/Akt pathway that regulates mTOR and have a resulting cytotoxic or cytostatic effect.

It was also hypothesized that inhibiting the PI3K dependent pathway would decrease mTOR protein activity and that by measuring the activity of PKC and mTOR proteins, the effects of tamoxifen could be evaluated. In this study, tamoxifen was used not as hormonal therapy but as a potential protein kinase C inhibitor targeting the RA 1 Burkitt's lymphoma cell line, whose cells are estrogen receptor negative. This project investigated not only tamoxifen's effect but also its mechanism of action in estrogen receptor negative cancers.

To better understand tamoxifen's mechanism of action in the Ramos cell line, it was necessary to determine how tamoxifen may inhibit protein kinase C. Recent studies have suggested that tamoxifen causes oxidative stress that inhibits the activity of protein kinase C [3]. Thus the cells were also pretreated with an antioxidant that neutralizes oxidative stress and tamoxifen's effect on antioxidant pretreated Ramos cells was observed. It was also crucial to determine whether any subsequent alteration in cell viability was due to apoptosis, cytotoxicity or a decreased rate of growth.

2 Procedure and Methods

2.1 Cell culture

Ramos (CRL- 1923), a sporadic Burkitt's lymphoma cell line, was obtained from the American Type Culture Collection (ATCC, Manassas, Virginia, USA). Cells were maintained in RPMI-1640 medium (Invitrogen, Carlsbad, California, USA) supplemented with L-glutamine (Invitrogen) and 10% FBS (Invitrogen), and incubated at 37ºC with 5% CO_2.

2.2 Tamoxifen Treatment

The Ramos cells were treated with various concentrations of tamoxifen (1-25µM) in DMSO (Sigma, St. Louis, Missouri, USA). Treatment time intervals were dependent upon protocol.

2.3 Assays

Rapamycin pretreated Cell viability assay

Numerous pathways that also affect cell survival are closely linked to the PKC/Akt/PI3k pathway. In order to confirm that any alteration in Ramos cell viability was due to specific inhibition of the PKC/ Akt/ PI3k pathway and mTOR protein by tamoxifen, Ramos cell viability was measured after administration of different concentrations of tamoxifen (1-25µM) along with a pre-treatment of Rapamycin (Sigma), an mTOR inhibitor (5nM).

L-Carnosine pretreated cell viability assay

To determine whether tamoxifen inhibits PKC through oxidative stress, cells were pretreated with the antioxidant, L-carnosine (10-50 µM) in water, in an attempt to neutralize the proposed oxidative stress caused by tamoxifen (5µM). Cell viability was then measured.

Cell viability assay

CellTiter 96® AQueous One Solution Cell Proliferation MTS Assay (Promega, Madison, Wisconsin, USA) was used to determine cell viability. After tamoxifen treatment, 15 µL of room temperature CellTiter 96® AQueous One Solution was added into each well of the 96-well assay plate containing Ramos cells seeded at 60,000 cells/well in 100µL of culture medium. The 96-well assay plate was incubated for 3 hours at 37ºC in a humidified, 5% CO2 atmosphere. Absorbance was recorded at 490nm using a microplate reader (BioTek ELx808; Winnoski, Vermont, USA).

Preparing lysates

Phosphate buffered saline (Invitrogen), lysis buffer (Promega), and protease inhibitor cocktail (Sigma St. Louis, Missouri, USA) were placed on ice. For each concentration of tamoxifen, 1 x 10^7 cells were transferred to a 15mL conical tube. The cells were then spun at 1200 rpm for 7 minutes to pellet. Cells were then washed with 1.5mL of ice cold 1x PBS. Cells were moved to a microcentrifuge tube and re-centrifuged at 1200 rpm for 7 minutes. PBS was decanted. 1mL of lysis buffer was added (10 µL of protease inhibitor cocktail was added just prior to lysis) for 10 minutes on ice. Cells were centrifuged at 13,000 rpm for 15 minutes and the clear supernatant was moved to a pre-chilled microcentrifuge tube. Samples were stored at -80°C.

PKC Kinase Activity, Phospho-mTOR Assay ELISA

The PKC Kinase Activity Kit (EKS- 420A) was purchased from Assay Designs (Butler Pike, Pennsylvania, USA); Phospho-mTOR ELISA (DYC1665-2) was obtained from R&D Systems (Minneapolis, Minnesota, USA). Assays were performed as per manufacturer's protocols. Briefly, 100µL of sample or standards were added to each well of 96 well pre-coated microplate for 24 h at 4°C. The plate was then aspirated and washed. The detection antibody was diluted to a working concentration and added (100µL) to each well, as per protocol. The plate was sealed and incubated at room temperature for 2 hours and washed. Streptavidin-HRP was diluted to the working concentration, and 100µL of the diluted Streptavidin-HRP was added to each well of the plate. The substrate solution was added and the plate was incubated at room temperature for 20 minutes. The plate was read using the microplate reader at 450nm after the stop solution was added.

Caspase 3/7Assay

A Caspase 3/7 assay was conducted to determine whether the decrease in the cell viability was due to apoptosis or to the necrotic toxicity of tamoxifen. 100µL of Caspase- Glo Reagent (Promega) was added to each well of the treated 96-well plate; seeded at 60,000 cells/well in 100µL of culture medium. The plate was covered with a seal and was

placed on a plate shaker at 300-500 rpm for 30 seconds. The plate was incubated at room temperature for 2 hours and the luminescence of each sample was measured (BioTek Synergy HT; Winnoski, Vermont, USA).

Cell Viability Assay of Combinatory Therapy of Tamoxifen+Methotrexate

The first line chemotherapeutic drug regimen used to treat BL includes drugs with many adverse side effects, one of which is methotrexate. Reports indicate that reducing mTOR may help sensitize cells to methotrexate [13]. It would be interesting to know if tamoxifen, which is hypothesized to decrease the phosphorylation of mTOR, can sensitize the cells to methotrexate enabling a lower dose of methotrexate in the regimen. For this reason, Ramos cells were treated with 5μM of tamoxifen in 24 well plate ($9.0x10^5$ cells/well). 24 hours after tamoxifen treatment, Ramos cells were then also treated with 100μM or 200μM of methotrexate. The cell viabiliy was determined using MTS assay after 24 hours of methotrexate incubation. To determine whether the combinatorial therapy of methotrexate+tamoxifen produced a synergetic effect, indicative of increased methotrexate sensitivity, the fractional response (percent death corrected for a maximum response) was calculated using the results of an MTS assay. The viability of the cells which were treated with tamoxifen (5μM) for 24 hours followed by treatment of methotrexate (100μM or 200μM) for 24 hours was compared to the sum of the fractional responses of the tamoxifen or methotrexate single therapies. The cells that received tamoxifen single therapy were incubated for 48 hours after tamoxifen treatment and cells that received methotrexate single therapy were incubated for 24 hours after treatment in synchrony with the combinatorial regimen. All cells were seeded at $9.0x10^5$ cells/well.

The following formula used to calculate the sum of the fractional responses of TAM and MTX single therapies [4]:
FA= fractional response to tamoxifen alone
FB= fractional response to methotrexate alone

$$Y=FA+FB-(FA*FB)$$

If the fractional response to the combinatorial therapy of TAM+MTX was greater than Y (additive response of tamoxifen and methotrexate single therapies), the combinatorial therapy was concluded to produce a synergetic effect.

2.4 Data Analysis

Data were analyzed using Excel (Microsoft, Redmond, Washington, USA). For the cell viability assays, samples were analyzed with n = 5. For caspase and ELISAs, triplicate samples were analyzed. The unpaired Student's t-test was used to determine statistical significance. Alpha was set at 0.05.

3 Results

Effect of tamoxifen on Ramos cell viability

Ramos cell viability decreased as the concentration of tamoxifen increased (Fig. 1) in a dose-response fashion. The decrease in cell viability was statistically significant ($p<0.05$) for all concentrations of tamoxifen (1-25 μM). This significant decrease in cell viability highlights tamoxifen's potential as a novel BL treatment.

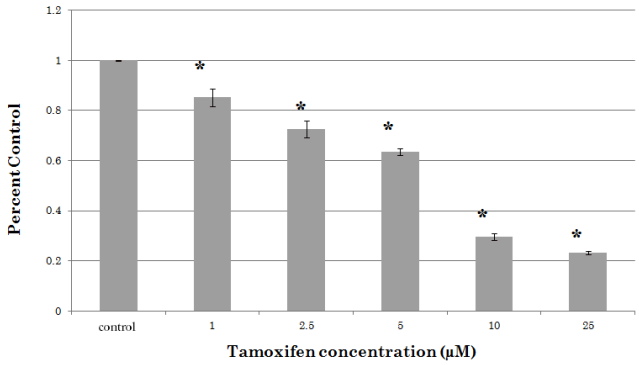

Fig 1. Effects of tamoxifen on Ramos cell viability. Increasing tamoxifen concentrations decreased Ramos cell viability in dose-response fashion. Bars are means ± standard deviation(SD) (n=5). *($p < 0.05$) compared with the solvent control.

Effect of tamoxifen on PKC kinase activity

To evaluate the effect of tamoxifen as a PKC inhibitor, PKC kinase activity was assessed in the presence of tamoxifen at 1-25 μM. The results obtained through ELISA (Fig. 2) show that at higher concentrations, tamoxifen reduced the PKC activity with statistical significance ($p< 0.05$). This data confirms tamoxifen's ability to inhibit PKC.

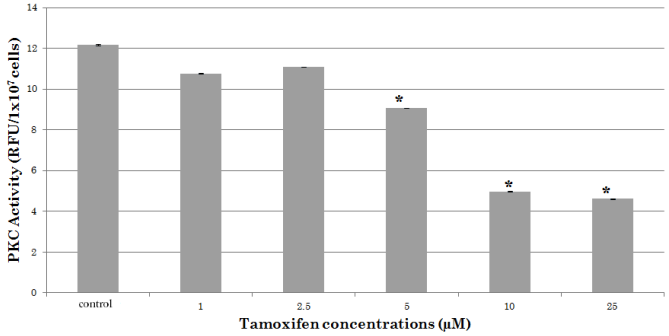

Fig. 2. Effects of tamoxifen on PKC kinase activity. PKC activity decreased significantly in cells treated with higher concentrations (5, 10, 25 μM)of tamoxifen. Bars are means ± SD (n=3). *($p < 0.05$) compared with the solvent control.

Effects of tamoxifen on Phosphorylation of mTOR

The phosphorylation of mTOR was measured using a phospho-mTOR ELISA. The level of phosphorylated mTOR indicative of mTOR activity was inversely correlated with the tamoxifen concentration with statistical significance ($p<0.05$) (Fig. 3). As the concentration of tamoxifen increased, the level of phosphorylated mTOR decreased in a dose-response fashion.

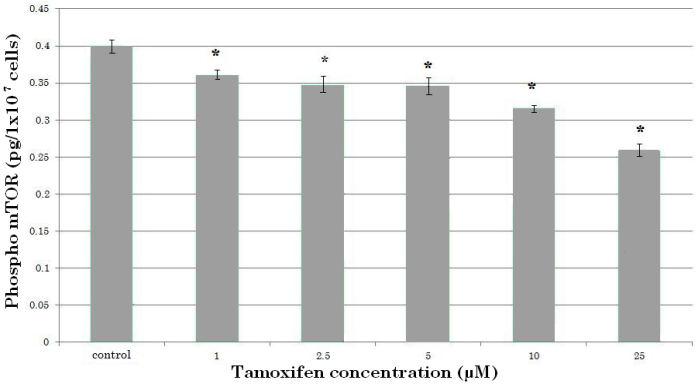

Fig. 3. Effects of tamoxifen on phospho-mTOR. As the concentration of tamoxifen increased, phosphorylated mTOR decreased. Bars are means ± SD (n=3). * ($p < 0.05$) compared with the solvent control

Effect of L-carnosine on Ramos cell viability after tamoxifen administration

To determine whether tamoxifen inhibits PKC through oxidative stress, Ramos cells were pretreated with L-carnosine, an antioxidant, before treatment with tamoxifen (5µM). If tamoxifen does inhibit PKC through oxidative stress, L-carnosine should, to some extent, negate tamoxifen's ability to cause PKC inhibition with corresponding reduced cell viability. The viability of L-carnosine pretreated cells after tamoxifen (5µM) administration increased with the concentration of L-carnosine (Fig. 4). The increase in the L-carnosine pretreated Ramos cell viability was statistically significant at 40µM and 50µM of L- carnosine when compared to cell viability of control. This observation suggests that tamoxifen may inhibit PKC through oxidative stress because tamoxifen's anti-apoptotic effect was negated by the pretreatment with L-carnosine.

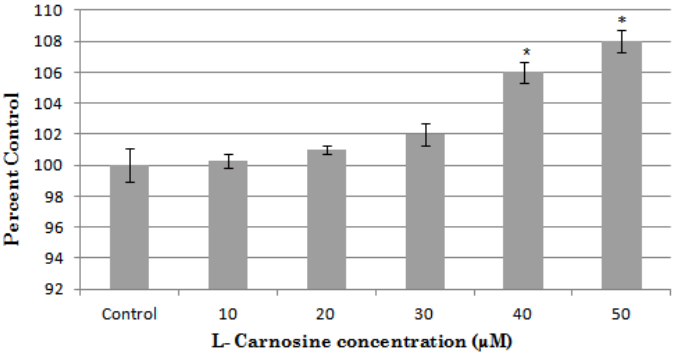

Fig. 4 Viability of L- carnosine pretreated Ramos cells after tamoxifen administration. The viability of tamoxifen-treated (5µM) Ramos cells increased as the L-carnosine concentration increased. Bars are the means ± SD (n=5). *($p < 0.05$) compared with the control.

Effect of tamoxifen on caspase 3/7

The Caspase 3/7 Assay was conducted to confirm that decrease in Ramos cell viability was not due to tamoxifen toxicity but induction of apoptosis. Since caspase 3/7 is considered an effector apoptotic enzyme, its activity should increase if the cells are progressing through programmed cell death. The graph (Fig. 5) shows that activity of caspase 3/7 increased as the concentration of tamoxifen increased. The result of the assay demonstrates that the decrease in cell viability was not due to tamoxifen toxicity, but due to the promotion of apoptosis.

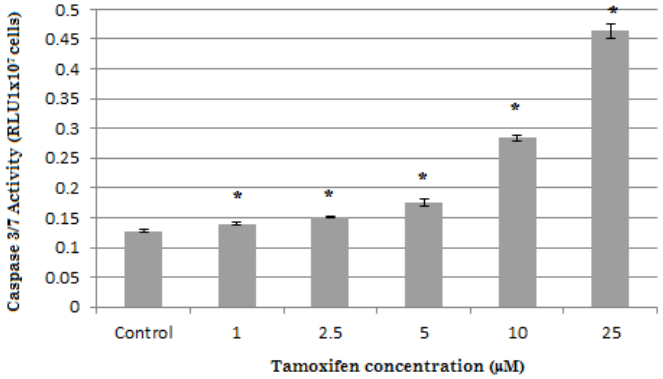

Fig. 5. Effects of tamoxifen on caspase 3/7. Activity of caspase 3/7 per cell increased as the tamoxifen concentration increased. Bars are the means ± SD (n=5). *($p < 0.05$) compared with the solvent control.

Effect of Rapamycin on Tamoxifen treated Ramos

Numerous other pathways that are linked to PKC/PI3k/mTOR pathway also lead to cell apoptosis. In order to confirm that tamoxifen induced apoptosis by inhibiting mTOR, the cells were pretreated with rapamycin, an inhibitor of mTOR, before being treated with tamoxifen. Viability of these cells was compared to that of the control treated with rapamycin alone (Fig. 6). The graph shows that tamoxifen (1µM -5µM) was not able to induce a significant increase in apoptosis in the rapamycin pretreated cells as the pathway was already inhibited (mTOR already inhibited by rapamycin). This data strongly supports the hypothesis that tamoxifen induces cell apoptosis in Ramos cells by inhibiting mTOR. At 10µM and 25µM the tamoxifen administration after rapamycin pretreatment caused a trend in increased apoptosis, but this trend did not demonstrate an additive effect when considering the apoptosis caused by tamoxifen alone (Fig.1) and rapamycin alone. This also suggests tamoxifen's effect

on the mTOR pathway.

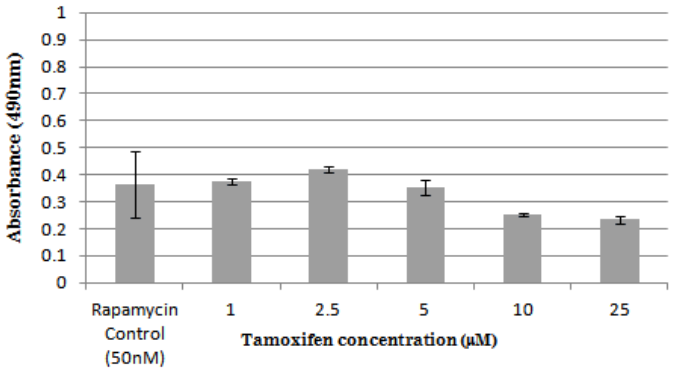

Fig. 6. Effects of tamoxifen on rapamycin-treated Ramos cell viability. The cell viability after administration of 50 nM of rapamycin and subsequent tamoxifen (1μM- 25μM) did not differ statistically. Bars are the means ± SD (n=5). * ($p < 0.05$) compared with the control.

Effect of Tamoxifen+Methotrexate Combinatorial Therapy

In order to determine whether tamoxifen is able to increase the cell's sensitivity to methotrexate, the fractional response (percentage of cell death measured by MTS assay) to tamoxifen (TAM) and methotrexate (MTX) single therapies versus that of TAM+MTX combinatorial therapy was evaluated. The cells treated with the combinatorial therapy were pretreated with TAM for 24 hours then MTX for an additional 24 hours at different concentrations (100μM, 200μM). The graph (Fig. 7) demonstrates the synergistic effect of the tamoxifen+methotrexte combinatorial therapy at methotrexate concentrations (100μM, 200μM). This data supports that tamoxifen does increase the cell's sensitivity to methotrexate.

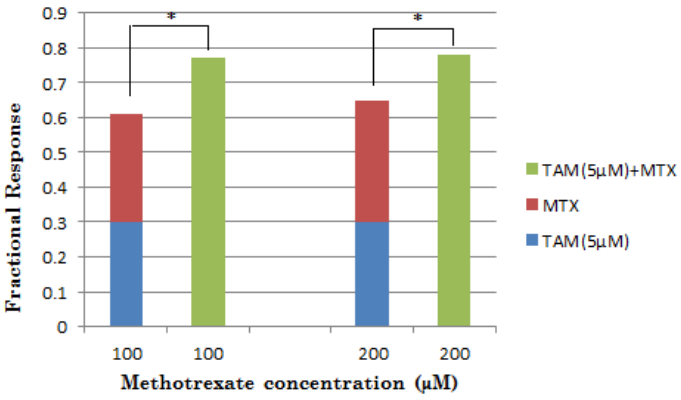

Fig. 7. Effects of Combinatorial therapy of tamoxifen and methotrexate.The combinatorial therapy of methotrexate and tamoxifen resulted in a significantly higher fractional response (percentage of cell death measured by MTS assay) compared to the additive response of tamoxifen and methotrexate single therapies ($p<0.05$). *($p< 0.05$) compared with the control

4 Discussion

The aim of this study was to investigate the mechanism of action of tamoxifen in estrogen receptor negative cancers, specifically Burkitt's lymphoma, using RA 1 (RAMOS), a Burkitt's lymphoma cell line. Tamoxifen is currently approved for use only in estrogen receptor positive breast cancers. Several studies have shown that tamoxifen functions as a protein kinase C inhibitor; thus tamoxifen's effect on PKC/PI3k/Akt pathway was investigated. Burkitt's lymphoma Ramos cell viability decreased with statistical significance across all concentrations of tamoxifen in a dose-response fashion after tamoxifen treatment (1-25μM). The activity of protein kinase C decreased in a dose-responsive fashion at higher concentrations of tamoxifen ($p<0.05$), showing that tamoxifen does inhibit PKC in estrogen receptor negative cells. Inhibition of PKC and PI3K/Akt pathway by tamoxifen decreased the phosphorylation of mTOR ($p<0.05$), the downstream target of PKC/PI3K/AKT pathway that is responsible for cell survival and proliferation. The results of the cell viability assay, mTOR ELISA, and PKC ELISA suggest that tamoxifen was able to effectively reduce Ramos cell viability by inhibiting PKC and its downstream target protein, mTOR.

The question then arose, how does tamoxifen reduce PKC activity. Recent studies proposed that tamoxifen inhibits PKC by causing oxidative stress, a condition where highly reactive free radicals cause damage to proteins, membranes, and genes. Antioxidants counteract oxidative stress by neutralizing free radicals. In order to determine whether tamoxifen causes PKC inhibition by oxidative stress, Ramos cells were pretreated with L- carnosine, an antioxidant, before tamoxifen treatment. The antioxidant was able to prevent cell apoptosis caused by tamoxifen thus it was concluded that tamoxifen inhibits PKC by causing oxidative stress.

Caspase 3/7 is an effector apoptotic enzyme, and its activity increases as the rate of apoptosis increases. Its activity is not expected to increase if the cell viability decreases simply due to tamoxifen toxicity, conversely the activity is expected to increase if the cell viability decreases due to inhibition of a protein or pathway essential to cell growth and survival. The luminescence intensity in the Caspase 3/7 Assay demonstrated that cell death was due not to the cytotoxicity of tamoxifen, but to the promotion of apoptosis through the upregulation of caspase 3/7 activity. With numerous other pathways that are closely related to PKC/Akt/mTOR pathway, it is difficult to assert that the decrease in Ramos cell viability was specifically due to mTOR inhibition. Therefore, the Ramos cells were pretreated with rapamycin, an mTOR inhibitor, before tamoxifen treatment. If the decrease in the cell viability was

due to mTOR inhibition, tamoxifen treatment should not cause any significant alteration in cell viability since mTOR (in rapamycin pretreated cells) is already inhibited by rapamycin. The lack of an incremental effect of tamoxifen on cells pretreated with rapamycin supported evidence that tamoxifen decreases Ramos cell viability by inhibiting the PKC/PI3k/Akt pathway and mTOR activity.

This possible mechanism of action of tamoxifen (Fig. 8) elucidated in this study highlights another approach to treating Burkitt's lymphoma and identifies the target pathway for chemotherapeutic purpose.

Fig. 8. Schematic pathway of tamoxifen's possible mechanism of action on Ramos (ER-) cell.

5 Application

Methotrexate is a standard component of the chemotherapy regimen for Burkitt's's lymphoma and it is delivered in high-dose; this can bring serious adverse effects such as hepatic, pulmonary, and renal abnormalities. At the same time, patients need high dose methotrexate because dihydrofolate reductase (DHFR) levels rise in cells exposed to methotrexate, causing "methotrexate insensitivity" [10]. A recent study shows that inhibiting the mTOR pathway lowers a cell's DHFR level, increasing its sensitivity to methotrexate [13]. For that reason, a cell viability assay comparing the effect of methotrexate and tamoxifen as single agents to that of combinatorial therapy (tamoxifen plus methotrexate) was carried out to determine whether tamoxifen could increase the cell's sensitivity to methotrexate. Increasing the cell's sensitivity to methotrexate can significantly lower the required dose of methotrexate, thus minimizing the possibility of adverse side effects, especially compelling in the pediatric demographic population. Data showed that tamoxifen was able to significantly increase the cell's sensitivity to methotrexate thus having the potential to lower the required "high-dose" methotrexate utilized in the treatment regimen of BL.

6 Conclusion

This project demonstrated that tamoxifen, which is currently used to treat estrogen receptor positive breast cancers, may have potential in the treatment of Burkitt's lymphoma and other estrogen receptor independent cancers that have an activated PKC/Akt/mTOR pathway. Tamoxifen's mechanism of action was proposed to be through PKC inhibition caused by oxidative stress. PKC inhibition led to a decrease in phosphorylated mTOR protein that is responsible for growth and survival. Tamoxifen's potential as a Burkitt's lymphoma therapeutic option was strengthened by the *in vitro* cell viability assay that showed significant decrease in Ramos cell viability after tamoxifen treatment and the Caspase 3/7 assay confirmed that this decrease was not due to tamoxifen toxicity, but due to apoptosis. These findings may have useful implications in the modification of the current treatment regimens for BL *in vivo*.

Furthermore, within the global healthcare arena, this study has significant implications. Burkitt's lymphoma is endemic, especially among children in the African countries, accounting for 36% of childhood cancers and 70% of childhood lymphomas. Most patients in these low-resource countries cannot afford the elaborate regimens available in the United States to treat this disease and treating BL patients with high-dose methotrexate is difficult in Africa due to lack of supportive care during chemotherapy. This demographic population needs a novel treatment that is effective, safe, and inexpensive. Tamoxifen has been used as a breast cancer therapy for many years and its safety and potential adverse effects are well documented. Moreover, it is more economical than most of other chemotherapeutic drugs in the regimens used to treat Burkitt's lymphoma. Tamoxifen's ability to increase the cell's sensitivity to methotrexate may significantly reduce necessary "high dose" methotrexate, hence the toxicity of the chemotherapy, when given in combination.

In conclusion, this study shows tamoxifen's ability to induce cell death in ER- cancer cells by inhibiting mTOR through suppression of the PKC/PI3k/Akt pathway and elucidates tamoxifen's mechanism of action in the treatment of Burkitt's lymphoma. Tamoxifen may therefore have potential value as a novel treatment for not just Burkitt's lymphoma, but other estrogen receptor independent cancers with an activated PKC/PI3k/Akt pathway.

Acknowledgments

The authors wish to thank Dr. Danie Kotze for his support and Bergen County Academies for its sponsorship.

References

[1] Ball L. SERM- responsive regulatory elements are differentially regulated by tamoxifen and raloxifene with ER alpha and ER beta. University of California, 121.

[2] Banthia V, Jen A, Kacker A. (2003). Sporadic Burkitt's Lymphoma of the head and neck in the pediatric population. Pediatr Otorhinolaryngol. 67 (1): 59-65.

[3] Ferlini C, Scambia G, Marone M, Distefano M, Gaggini C, Ferrandina G, Fattorossi A, Isola G, Benedetti P, Mancuso S. Tamoxifen induces oxidative stress and apoptosis in oestrogen receptor- negative human cancer cell lines. Br J Cancer. 257-63.

[4] Greco WR, Bravo G, Parsons JC. The search for synergy: a critical review from response surface perspective. Pharmacol Rev 1995;47:331–85.

[5] Gundimeda U, Chen Zhen- Hai, Gopalakrishna Rayudu. Tamoxifen Modulates Protein Kinase C via Oxidative Stress

in Estrogen Receptor- negative Breast cancer cells. The Journal of Biological Chemistry. 13504-13514.

[6] Helen J, Mackay J, Christopher J. Targeting the protein kinase C family: are we there yet? Nature Reviews Cancer 554-562.

[7] Hiroshi M, Masanobu T, Yuzuru Y, Mitsuhiko O, Takao S, Itoh T, Takashi K, Shozo N. Tamoxifen inhibits tumor cell invasion and metastasis in mouse melanoma through suppression of PKC/MEK/ ERK and PKC/PI3K/AKT pathways. 2008.

[8] Kawauchi K, Ogasawara T, Yasuyama M, Otsuka K, Yamada O. The PI3K/Akt pathway as a target in the treatment of hematologic malignancies. Anticancer Agents Med Chem. 550-9.

[9] Mori T, Sairenji T. Functional role of phosphatidylinositol 3- kinase/ Akt pathway on cell growth and lytic cycle of Epstein- Barr virus in the Burkitt's lymphoma cell line, P3HR-1. Virus Genes: 327-34.

[10] Saydam G, Celikkaya H, Cole P, Bertino J, Emine A. mTOR inhibition leads to increased sensitivity to methotrexate. Proc Amer Assoc Cancer Res, Volume 46, 2005.

[11] Straus D. Treatment of Burkitt's's lymphoma in HIV-positive patients. Biomedicine & Pharmacotherapy Volume 50, Issue 9, 447-450.

[12] Suzuki E, Umezawa K, Bonavida B. Rituximab inhibits the constitutively activated PI3K/Akt pathway in B-NHL cell lines: involvement in chemosensitization to drug-induced apoptosis. Oncogene. 6184-6193.

[13] Teachey D, Sheen C, Hall J, Ryan T, Brown V, Fish J, Reid G, Seif A, Norris R, Chang Y, Carroll M, Grupp S. mTOR inhibitors are synergistic with methotrexate: an effective combination to treat acute lymphoblastic leukemia. Blood. Sep 1; 2020-3.

[14] Vivanco I, Charles L. The phosphatidylinositol 3-kinase- AKT pathway in human cancer. Nature Reviews Cancer2, 489- 501.

[15] Zhou H, Huang S. (2010). Role of mTOR Signaling in Tumor Cell Motility, Invasion, and Metastasis. Curr Protein Pept Sci.Dec 29.