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Mucoxin (Acetogenin) Reduces Proinflammatory Cytokines in Breast Cancer

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Mucoxin is a potential compound used as an anticancer agent. Mucoxin induced apoptosis and inhibit proliferation in T47D breast cancer cells line. This study aims to determine the effect of mucoxin on proinflamatory cytokine in breast cancer. proinflamatory cytokine play important role in the development and metastasis of cancer cells. Breast cancer cell line MCF-7 were grouped into five groups referred to mucoxin doses assays, they are 0 ng/mL; 0.1 ng / mL; 0,5 ng/mL; 1 ng/mL; 5 ng/mL with three replication of each. Mucoxin was given for 48 hours. The levels of IL 6 and TNF-α assayed using ELISA methods. The results showed mucoxin decreases IL 6 levels in all treatment doses, but was not significant. Mucoxin also decreases TNF-α levels, with a significant reduction occurring at doses of 1 ng/mL and 5 ng/mL. It is suggested that mucoxin has potent to inhibit proinflammatory cytokines that play a role in the development and metastasis of breast cancer.

Keywords: Acetogenin, IL 6, mucoxin, MCF-7, TNF-α.

Breast cancer is the most common cancer in women and is the second leading cause of death after cervical cancer.[1,2] Breast cancer can be treated with various treatment methods, such as surgery, chemotherapy, hormone therapy, radiation therapy, and immunological therapy.[3]One of the most common treatment methods is chemotherapy. Chemotherapy is a treatment carried out by giving anticancer compounds to suppress cell proliferation and trigger apoptosis.[4]Unfortunately, chemotherapy has various side effects and some cancer cells begin to be resistant to chemotherapy.[5]

Based on these facts, there are currently many new anti-cancer drugs being developed. One goal of that is to increase the sensitivity of pre-existing therapies. Acetogenin and its derivatives, such as mucoxin, are promising anti-cancer compounds. Mucoxin is a non-classical acetogenin compound which was first isolated from Rollinia mucosa leaf extract by McLaughlin in 1996.Based on the results of our previous study, this compound was able to induce apoptosis and inhibit the proliferation of T47D breast cancer line cells.[6]

One of the causes of the high mortality rate of breast cancer is its ability to metastasize to various tissues that are far from their origin tissues.[7] Previous studies have shown the involvement of various proinflammatory cytokines, such as tumor necrosis factor α (TNF-α) and interleukin 6 (IL 6),

on the progression of breast cancer.[8,9] Therefore, this proinflammatory cytokine has the potent to be a therapeutic target for new anticancer drugs to prevent metastatic cancer.

On the other hand, the acetogenin group compound is known to have the ability as an anti-inflammatory and anti-angiogenesis in cancer cells.[10,11] Given the mucoxin potent as an anti cancer, it is interesting to assess its ability, to reduce proinflammatory cytokines that promote growth and metastasis in breast cancer.The proinflammatory cytokines measured were IL 6 and TNF-α. The results of this study are expected to provide information about the ability of mucoxin in inhibiting proinflammatory cytokines that play a role in the growth and spread of cancer cells.

METHODS

Material

Mucoxin was obtained from Angene International Limited with ID AG-E-32919 and CAS No. 183195995. Human breast cancer cell line MCF-7 was a collection from the Cytogenetics laboratory and Cell Culture Unpad Faculty of Medicine..

Experimental design

This study used a randomize block design. Breast cancer cell line MCF-7 were grouped into five groups referred to doses assays, they are 0 ng/mL; 0,1ng/mL; 0,5 ng/mL; 1 ng/mL; 5 ng/mL with three replication of each and exposed to mucoxin for 48 hours.

Cell Culture

The cells were grown in Roswell Park Memorial Institue Medium (RPMI1640). This media supplemented with 10% Fetal Bovin Serum (FBS) Gibco™ (Thermo Fisher Scientific Cat. 26140-079) and 0,2 mL bovine insulin (Sigma Aldrich Cat. No. 15500 and CAS RN 11070-73-8) at 37° C in 5% CO_2. Thawing process performed in waterbath at 37° C for 2-4 min. Then, $5x10^4$ cells cm^{-2} was taken into T-flask and incubated at 37° C in CO_2 5%. When cells density reached 80% confluent, the trypsinization was done using 0,25% trypsin+0,53mM EDTA solution and then subcultured into new culture vessels, also incubated at 37° C in CO_2 5%. Afer two times passaging, the MCF-7 cells ready to be treated.

Mucoxin Treatment

Mucoxin exposed to MCF-7 cell line culture, was done after the cells reached confluent 80% 90%, cell morphology was still good and it was confirmed that there was no contamination and fixed pH at each medium.Mucoxin preparations were dissolved in 0.1% DMSO solution. Furthermore, dilution is made at a dose of 0 ng / mL (K); 0.1 ng / mL (P1); 0.5 ng / mL (P2); 1 ng / ml (P3); 5 ng / mL (P4). Then, into each well, mucoxin was diluted according to the treatment doses and incubated for 48 hours. After the treatment time was reached, the culture medium of the well was taken and the TNF-α and IL 6 levels are examined.

TNF- α Assays

TNF-α examination was performed using the ELISA method. Into the wells that have contained TNF-α antibodies, culture media were included from the cell line treatment and incubated for 1 hour.Furthermore, washing was done to remove non-specific antibodies that were not bound. The second antibody was added which had been given horse radish peroxidase (HRP) enzyme and incubated. After being incubated for 30 minutes, the MTB substrate was added to the well and incubated for 30 minutes. The blue color that appears was a sign of TNF-α antibody binding. The next step was the addition of the stop solution and observe the color changes that occur from blue to yellow and measured by a spectrophotometer at a wavelength of 405 nm.

IL 6 Assays

IL 6 examination was performed using the ELISA method. Into the wells that have contained IL 6 antibodies, culture media from the cell line treatment was incubated for 1 hour. Furthermore, washing was done to remove non specific antibodies that were not bound. The second antibody was added which had been given horse radish peroxidase (HRP) enzyme and incubated. After being incubated for 30 minutes, the TMB substrate was added and incubated for 30 minutes. The blue color that appears was a sign of the antibody binding of IL 6. The next step was the addition of the stop solution and observe the color changes that occur from blue to yellow and measured by a spectrophotometer at a wavelength of 405 nm.

Statistical Analysis

Comparison of mean values between treatment are presented as mean±SD and analyzed using ANOVA followed by LSD test with a 95% confidence level.

RESULTS

Effects of Mucoxin on IL 6

The results of the measurements that have been carried out, show a decrease in IL 6 levels in the group given mucoxin when compared with the control. The highest mean IL 6 levels were found in the control group, which was 3.87 pg / mL. The level then decreases with increasing dose of mucoxin exposed. However, this decreases was not significant (p>0,5) (Table 1).

Effects of Mucoxin on TNF-α

Same with the measurement of IL 6 levels, the results of measurements of TNF-α levels also showed a decrease in the group given mucoxin, when compared to controls. The highest mean TNF-α level was found in the control group, which was 132.43 pg/mL. The level then decreased significantly along with the increase in the dose of mucoxin given (p <0.05) (Table 2).

DISCUSSION

Mucoxin is an acetogenin derivative which has the potential effect as an anticancer agent. Our previous research shows that these compounds can trigger apoptosis and inhibit the proliferation of T47D breast cancer line cells.[6]

One of the causes of deadly breast cancer is because of its ability to metastasize and spread to various tissues that are far from their original tissues. Research shows the ability of cancer cells to metastasize and spread, associated with the involvement of various proinflammatory cytokines, such as IL 6 and TNF-α.

Interleukin 6 (IL 6), is a proinflammatory cytokine that contributes to the invasion and spread of cancer cells. IL 6 induces proliferation and increases the aggressiveness of cancer cells.[12]

In breast cancer patients, IL 6 levels are known to be higher than in non-breast cancer patients. High IL 6 levels, also associated with an increase in the number metastases location and poor prognostics in breast cancer patients.[13]

There are several mechanisms of IL 6 involvement in metastasis and tumor spread, including IL 6 enhancing matrix metalloproteinase (MMPs) expression which is able to degrade extra cellular matrix components. IL 6 also increases the regulation of various adhesion molecules such as ICAM-1 and ELAM-1, thus facilitating tumor attachment to endothelial cells.[14-16]

On in vitro studies, IL 6 has been shown to stimulate growth and MCF-7 cell invasion ability. Overexpression of IL-6 in MCF-7 cells induces the formation of epithelial mesenchymal transition (EMT) which promotes cancer cell invasion ability.[17]

The results of our research, showed that administration of mucoxin for 48 hours was able to reduce IL 6 levels of breast cancer cells, although not significantly different (Table 1). However, there was a tendency to decrease IL 6 levels by increasing the dose of mucoxin given. These results were still in accordance with previous studies of other acetogenin compounds which show that acetogenin can reduce IL 6 levels in cancer cells H_{22}.[18]

Table 1. The mean level of IL 6 in the MCF-7 breast cancer cell line treated withmucoxin for 48 hours with different concentrations

Mucoxin Concentration	IL 6 Level (Mean±SD)	p value
0 ng/mL	3,88 ± 1,32	0,061
0,1 ng/mL	3,83 ± 0,60	
0,5 ng/mL	2,97 ± 0,78	
1 ng/mL	2,80 ± 0,36	
5 ng/mL	2,01 ± 0,19	

Table 2. The mean level of TNF-α in the MCF-7 breast cancer cell line treated withmucoxin for 48 hours with different concentrations

Mucoxin Concentration	TNF-α Level (Mean±SD)	p value
0 ng/mL	3,88 ± 1,32[a]	0,001
0,1 ng/mL	3,83 ± 0,60[a]	
0,5 ng/mL	2,97 ± 0,78[a]	
1 ng/mL	2,80 ± 0,36[b]	
5 ng/mL	2,01 ± 0,19[c]	

Mean±SD values followed by the same superscript are not different at α=0,05 by LSD test

Mucoxin decrease IL 6 levels was thought caused the inhibition of one of the IL 6 activation pathways in the cell. IL 6 is a cytokine produced by various types of cells during the infection phase, trauma, changes in immune conditions and malignant conditions. The action mechanism of IL 6 on cells, is carried out through the bond between IL 6 and its receptor (IL-6R), found in the cell membrane. Mucoxin compounds, known to induce apoptosis through the activation of Bax proteins that cause changes in cell membrane permeability. This change in permeability is thought to change the form of IL 6 receptors on the membrane surface, so IL 6 activation becomes disrupted. In addition, IL 6 activation also involves the Janus kinase (JAK) pathway.[16] Acetogenin compounds are known to inhibit JAK activation.[18]Inhibition of this compound, will reduce the expression of IL 6 in the cell.

IL 6 is known to be involved in STAT3 activation in breast cancer cells.[17]The existence of pathways involving IL 6 / JAK / STAT3 causes cells to develop into neoplastic, protect cells from apoptosis and cause cancer cell resistance from chemotherapy drugs.[18]The ability of mucoxin to reduce IL 6 causes a disturbance in the IL 6 / JAK / STAT3 pathway, thus inhibiting cell changes to neoplastic, encouraging apoptosis and decreasing the ability of cancer cells to form EMT that is needed for invasion and metastasis.

Besides IL 6, proinflammatory cytokines that also play a role in the process of metastasis and the spread of cancer cells are TNF-α. This TNF-α, expressed very high in breast cancer. TNF-α in large quantities acts as an anti-tumor, but in low doses, this cytokine is known to increase tumor growth and spread.[19]In addition, TNF-α also plays a role in angiogenesis which is needed for cancerous tissue to develop in new areas.

In the breast cancer cell line, TNF-α increases cell proliferation through increased NF-kB caused byincreased cyclin D1 expression. TNF-α is also known to increase the effect of estrogen in causing cell proliferation. In the MCF-7 cell line, TNF-α promotes migration, invasion and resistance to chemotherapy drugs.

The role of TNF-α in promoting the growth, metastasis and spread of breast cancer is done through several mechanisms, including through the induction of various mediators that play a role in the growth and metastasis of breast cancer. In the invasion process, TNF-α plays a role in increasing the expression of matrix metalloproteinases (MMPs) and dipeptidyl peptidases (DPPs).[19]In addition, TNF-α also plays an important role in EMT formation.

The results of our research have shown that administration of mucoxin can significantly reduce TNF-α levels of cancer cells. Decreased levels of TNF-α along with increased doses of mucoxin given (Table 2). These results are consistent with previous studies of other acetogenin compounds, which were extracted from soursop leaves (Annona muricata), which showed that these compounds were able to reduce TNF-α levels.[20]

TNF-α levels decreased by mucoxin can occur through several mechanisms, including a decrease in transcription from genes encoding TNF-α.This decrease in transcription is thought to be influenced by increased gene transcription that has an opposite effect with TNF-α, such as p53 and decreased transcription of genes associated with TNF-α, such as cyclin D1. On cell line, TNF-α, along with cyclin D1, is known to increase proliferation.Whereas p53 decreases cyclin D1 activity, so its increase will decrease cell proliferation. Based on previous research, mucoxin has been shown to influence the transcription of these genes.[6]

In addition to transcription factors, decreased TNF-α by mucoxin, can also occur due to epigenetic mechanisms. Mucoxin is thought to be able to inhibit histone acetylation in the TNF-α locus by increasing histone deacetylase. Increased histone acetylation, associated with increased cell ability to produce TNF-α, so inhibition of histone acetylation will reduce TNF-α production. In addition, mucoxin is also thought to play a role in DNA methylation in the TNF-α gene locus. The presence of methylation in the TNF-α locus, causing a decrease in TNF-α production by cells.[21]

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