RESEARCH ARTICLE

The Effect of NSAIDS and Oral Hypoglycemic Agent on Leukemia and Lymphoma Cell Lines

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ABSTRACT

Cancer is regarded as global burden and a serious health challenge. Its occurrences increased due to population aging and the prevalence of risk factors. This study investigated the effect of metformin and aspirin on two cell lines SR (diffuse large B cell lymphoma) and NB4 (promyelocytic leukemia). The effect of metformin as monotherapy on SR was 28.43% viable, while on NB4, only 44.4% were viable. However, the effect of aspirin on SR and NB4 cell lines were decreased; the percentage of cell viability were 27.2 and 41.2%, respectively. In conclusion, there is significant effect of metformin and aspirin on SR cell line and only mild effect on NB4. These may be useful drugs in old diabetic patients with diffuse large B cell lymphoma that may reduce the chemotherapy dose.

Keywords: NSAIDS, LCS, Oral hypoglycemic drug.

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INTRODUCTION

Cancer is regarded as global burden and a serious health challenge.[1,2] Cancer occurrence increases due to population aging and the prevalence of risk factors.[3,4] Acute myeloid leukemia (AML) is a set of neoplastic blood disorders depicted by the accumulation and proliferation of immature hematopoietic cells in blood and bone marrow. It's almost 20 and 80% of acute leukemia in children and adults, respectively. The AML incidence progressively increases with age.[4] Over the age of 65, the prevalence is almost 30 times the prevalence of AML in children. Aspirin is the most common drug used in medicine for over a century.[5] The therapeutic advantages are antipyretic, analgesic and anti-inflammatory effects.[6] It targeted both cyclooxygenase 1 and 2 (COX-1 and COX-2).[7,8] As noticed by epidemiological data have shows a dramatic reduction in the incidence of cancer in patients taking low dose of aspirin daily[8-10] aspirin is regarded as a powerful chemopreventive agent. There is evidence that platelets functions linked to tumorigenesis and metastasis.[11,12] Aspirin has both biochemical and biological effect on platelets.[13,14] Metformin is biguanide derivative antidiabetic used for one century.[15] According to many epidemiological studies, this drug can reduce the risk of different malignancies and may also improve prognosis.[16] Unfortunately, many studies on hematological malignancy reported heterogeneous results.[17,18] Therefore, further studies upon the metformin and aspirin effect on acute myeloid leukemia and immunoblastic lymphoma cell lines as an *in-vitro* study is suggested to explore their effect on those two hematological cell lines. This study is attentive to investigating the effect of metformin (oral hypoglycemic drug) and aspirin (NSAID) separately on leukemia and lymphoma cell lines compared the outcomes.

MATERIALS AND METHODS

This study was conducted at the Iraqi Center of Cancer and Medical Genetics Research (ICCMGR) 2021.

Drugs Preparation

Metformin drug (1,1-dimethylbiguamidehydrochlloride) was obtained from Sigma Chemical Company while aspirin drug (acetylsalicylic acid) was obtained from CDH company. Dimethyl sulfoxide (DMSO) solvent was used to dissolve metformin and dilute in culture medium with serial dilution of concentrations 39, 78.1, 156.2, 312.5, 625, 1250, 2500, and 5000 μg/mL which were freshly prepared. Phosphate buffer saline (PBS) was also used to dissolve aspirin and diluted with culture medium to prepare the following concentrations 39, 78.1, 156.2, 312.5, 625, 1250, 2500, and 5000 μg/mL.

Cell Culture

The effect of the metformin and aspirin were studied (*in-vitro* method) on a human lymphoma cell line SR, and

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promyelocytic leukemia cell line NB4 (provided from the experimental therapy department/ICCMGR). These cell lines were maintained in minimal essential media (GIBCO chemicals) supplemented with 10% fetal bovine serum (FBS), and used in cytotoxicity assay.

Preparation of Cell line and Cytotoxicity

Method of Freshney, R.I, 2005 was followed with initial step of dispensing all growth medium from the cell sheet. Next, 2–3 mL of trypsin versine (U.S. Biological Co., USA) was added to the cell sheet and gently racked. After about 30 seconds most of the tyrosine-versene was dispensed off and then cells were incubated at 37°C for 10 minutes to be ensured all cells detached from the plate. Cells were further soaked by pipetting in growth medium.

Thus, 200 µL of cells in a growth medium with a concentration 1×10^4 were added to the sterile 96 well black plates. The 96 well plates were wrapped with a self-adhesive film, and lid placed on, and incubated for 24 hours at 37°C. When the cells are in exponential growth, (Lag phase), the medium was soaked and serial dilutions of metformin, mspirin and ketorolac drugs in serum free media (SFM) were added (for each concentration of each drug, six replicates were added). Then, the plates were incubated for another 72 hours at 37°C exposure time.

Next, supernatants were decanted off from well plates after each exposure period ended, while sterile conditions were maintained and 100 µL of crystal violate stain was added. After that, plates were covered and incubated for 20 minutes at 37°C and then plates were washed and allowed to dry. Finally, The optical density (OD) was measured at 450 nm using microplate reader.

The observed results were then compared with control which was incubated in the absence of the above drugs. Cell viability % was estimated using the following formula:

$$cell\ viability\ \% = \frac{mean\ OD}{control\ OD} \times\ 100\%$$

RESULTS

In order to assess the intrinsic effect of aspirin and metformin (NASAIDS) drugs, assays of leukemia and lymphoma cell viability with MTS were performed to determine the suitable concentration for an effective method to treat this type of cancer.

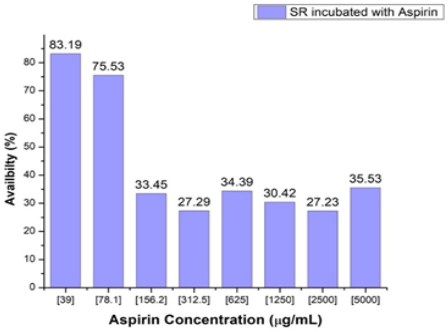

Figure 1: Cell viability of SR cell lines incubated with aspirin.

In this regard, SR cell lines were incubated with different concentrations of aspirin 39, 78.1, 156.2, 312.5, 625, 1250, 2500, and 5000 µg/mL and with metformin 39, 78.1, 156.2, 312.5, 625, 1250, 2500, and 5000 µg/mL as well. NB4 cell lines also were incubated with aspirin and metformin 39, 78.1, 156.2, 312.5, 625, 1250, 2500, and 5000 µg/mL concentrations for both drugs. The observed results showed that aspirin incubated with SR cell lines decreased cell viability from 83.19 to 27.23% when its concentration goes from 39 to 2500 µg/mL as shown in Figure 1. Moreover, SR cell lines decreased in viability from 68 to 28.43% when incubated with metformin from 39 to 5000 µg/mL concentrations, Figure 2. For NB4 cell lines, the results exhibited, that cell viability decreased from 94.61 to 41.18% when loading was doubled from 39 to 2500 µg/mL of aspirin concentrations (Figure 3). The same for NB4 cell lines after incubated with metformin were cell viability decreased from 79 to 45.8% when concentrations increased from 39 to 2500 µg/mL as shown in Figure 4.

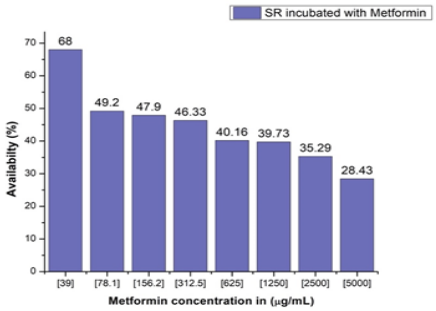

Figure 2: Cell viability of SR cell lines incubated with metformin.

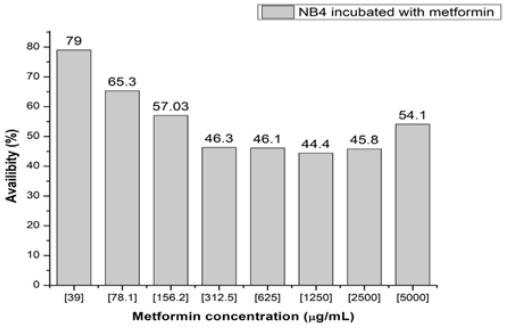

Figure 3: Cell viability of NB4 cell lines incubated with aspirin.

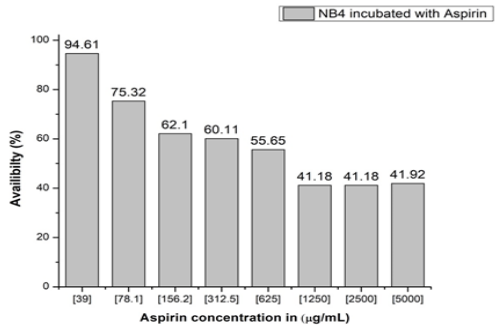

Figure 4: Cell viability of NB4 cell lines incubated with aspirin.

DISCUSSION

Lymphoma are malignant tumor of lymphoid tissue. Lymphoma are diagnosed as a malignant blood tumor with heterogenous course and different prognosion. Our data revealed significant effect of metformin on SR cell line. That represents diffuse large B cell lymphoma (DLBCL) which is the most frequent type of B cell lymphoma, as shown in Figure 2 that the effect is dose dependent and only 28–43% of cells are viable. These results were similarly identified by Shi *et al.,* that metformin-induced suppression of growth in B and T cells lymphoma. The effect is also dose dependent the incidence of NHL in patients on metformin is significantly lower than that with non-metformin antidiabetics.[19] However, Smyth *et al.,* demonstrate that metformin had a non-significant effect on survival in a population-based study on more than 66 years patients.[20] Also, Wang *et al.* did not identify any evident effect of metformin on DLBCL lymphoma in newly diagnosed patients.[21] Metformin has anticancer effect by both direct and indirect mechanisms. Metformin at molecular level inhibits mitochondrial respiration at the respiratory chain complex I. Radiotherapy using metformin is important in tumors deficient in p53 that are often resistant to chemotherapy. Also, metformin has antifolate effect that inhibits cancer cell growth. The indirect effect of metformin inhibits insulin and insulin-like growth factor-1 by lowering of glucose level and also insulin level. Insulin and insulin-like growth factor-1 stimulate tumorigenesis. Metformin has some effect on the inflammatory process that involves in tumor progression. Transcription factor inhibition leads to diminished production of proinflammatory cytokines. Also, metformin found to stimulate the immune response to malignant cells. Our study identified that is 44.4% viable cells at 1250 μg/mL. NB4 cell line represents acute myeloid leukemia promyelocytic (M3) with.[15-17] These results consistent with that identified by Ademar *et al.* that metformin in combination with Arac had an antileukemic effect. Metformin had synergistic to Arac that may be a therapeutic option for older patients that can't tolerate chemotherapy.[22] Also Huai *et al.* showed the synergistic effect of metformin with trans-retinoic acid that induced apoptosis after the differentiation of blast cells in cell line.[23] Moreover, Asik *et al.* demonstrate that paclitaxel with metformin-induced significant apoptosis in a leukemia cell line that could be a new combination therapy for acute promyltic leukemia.[24] Epidemiological and clinical studies have shown that aspirin could decrease the risk of many types of cancer like colorectal cancer, prostate, breast, skin and lung cancer.[24] Few studies showed the effect of aspirin on hematological malignancies like acute myeloid leukemia and (LCBL). As shown in Figure 2 aspirin effect on SR cell line at concentration 312.5 μg/mL the viable cells are only 27.29%. while the effect of aspirin on NB4 cell line is only 41.1% viable cells at a concentration of 1250 μg/mL. Aspirin has COX-1 and COX-2 effect and non-COX effect that induced apopitosis through activation of p38 MAP kinase and caspases activation. NAIDs, including aspirin, downregulated telomerase activity and cancer cell growth. Telomerase can be inhibited genlicaly or pharmacologically, limiting the capacity of leukemia stem cell self-renewal through arrest of cell cycle and activation of p53 apoptosis and DNA damage.[25]

CONCLUSION

There is strong evidence that platelets play a role in carcinogenesis and metastasis development. *In-vitro* study when soluble fibrin and malignant cells injected showed increased metastasis *in-vivo*. Also invitro study showed interaction between malignant cells and platelet. Also, platelet activation may increase proliferation of malignant cells *in-vivo* and *in-vitro* after incubation of tumor cells and platelets. Aspirin significantly decrease malignant cell proliferation *in-vitro* and *in-vivo*.[25,26] Aspirin also induces cytochrome C release from mitochondria, subsequently activating the caspases. This mechanism could play a part in the beneficial effect of aspirin in cancer prevention. Moreover, aspirin has an effect on migration cell proliferation and apoptosis that may have therapeutic targets for patients with hematological malignancies.

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