تعديل تأثير الادوية مضادة السرطان في المختبر باستخدام ميتافورمين على خطوط خلايا البشر السرطانية

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المستخلص: إن تأثير الأدوية مضادة السرطان يعتمد على ملائمتها الحيوية وسميتها المتدنية. الهدف من هذه الدراسة هو استخدام الميتافورمين كعلاج مساعد للأدوية السرطان الكيميائية بغرض تحسين مفعولها كمضادات لانتشار الخلايا السرطانية بالإضافة لتقليل سمية الأدوية السرطانية الكيميائية. دُوسِت هذه الخصائص عن طريق تأثير خلط الميتافورمين بالسيس بلاتين على الخلايا لتقليل الآثار الجانبية. لقد استنتج أن خليط الميتافورمين والسيس بلاتين يقلل من تأثير الأدوية السرطانية مثل سرطان الكبد مقارنة باستخدام الأدوية السرطانية لوحدها. وأن الدراسة توصي بفعالية خلط الأدوية السرطانية بالميتافورمين مع قلة سميتها في الحيوانات.

الكلمات المفتاحية: ميتافورمين، سيس بلاتين، خطوط الخلايا السرطانية

(a) Control

(b) Cisplatin

(c) Paclitaxel

(d) Metformin

(e) combined metformin & cisplatin (f) combined metformin & paclitaxel

Fig (4): Cell viability of HepG2 in (a) control and in response to (b) cisplatin; (c) paclitaxel; (d) metformin; (e) combined metformin & cisplatin and (f) combined metformin & paclitaxel.

Fig (2): Percentage of viability of HepG2 by using different concentrations of cisplatin alone or combined with metformin (M) after 48 hours.

Fig (3): Percentage of viability of HepG2 by using different concentrations of paclitaxcel alone or combined with metformin (M) after 48 hours.

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Fig (1): Percentage of viability of HepG2 by using different concentrations of metformin after 48 hours.

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treat cancer. Akt expression as anti-apoptotic biomarkers is recombined to be carried out to know the role and mechanism of the anti-proliferative of the cancer cells against cisplatin and metformin (24).

The IC_{50} of paclitaxel alone or paclitaxel combined with metformin at concentrations 62.5, 125, 250 and 500 $\mu\text{g/ml}$ were 569.5, 618.5, 412.5, 370.8, 304.3 $\mu\text{g/ml}$ in HepG2 cell lines. The IC_{50} of combined metformin at concentration 125, 250 and 500 $\mu\text{g/ml}$ showed a significant decreased in cell viability respectively relative to paclitaxel alone.

It was reported that both metformin and paclitaxel inhibited cell viability of MCF-7 and A549 cells, as related to vehicle treated cells, and the combined treatment was more effective than either treatment alone (13).

In conclusion metformin potentiates the effects of both cisplatin and paclitaxel in HepG2 cell line. Further studies are required to prove it. It is recommended study the efficacy of the combination of therapeutic drug with metformin in animal as promising with high efficacy and less toxicity.

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Results

The IC_{50} of metformin (62.5-1000 $\mu\text{g/ml}$) was 385.3 $\mu\text{g/ml}$ in HepG2 cell lines (Fig 1).

The IC_{50} of cisplatin alone (3.75-50 $\mu\text{g/ml}$) or cisplatin combined with metformin at concentrations 62.5, 125, 250 and 500 $\mu\text{g/ml}$ were 3.39, 3.234, 2.669, 2.402, 1.809 $\mu\text{g/ml}$ in HepG2 cell lines. The IC_{50} of combined metformin showed a significant decreased in cell viability respectively relative to cisplatin alone (Fig 2).

The IC_{50} of paclitaxel alone (7.5-120 $\mu\text{g/ml}$) or paclitaxel combined with metformin at concentrations 62.5, 125, 250 and 500 $\mu\text{g/ml}$ were 569.5, 618.5, 412.5, 370.8, 304.3 $\mu\text{g/ml}$ in HepG2 cell lines. The IC_{50} of combined metformin at concentration 125, 250 and 500 $\mu\text{g/ml}$ showed a significant decreased in cell viability respectively relative to paclitaxel alone (Fig 3).

The effect of cisplatin, paclitaxel, metformin, combined metformin & cisplatin and combined metformin & paclitaxel on cell viability of HepG2 showed in Fig 4.

Discussion

The most common conspicuous malignancy and cause of death in the world is hepatocellular carcinoma (HCC). The main treatment of patients with HCC is chemotherapeutic drugs such as cisplatin and doxorubicin (19).

Due to the resistance, side effects and toxicity of chemotherapeutic drugs were elucidated in patients with cancer, in this study the adjuvant metformin drug combined with cisplatin or paclitaxel was evaluated in three different human cell lines to increase the efficacy of cisplatin or paclitaxel and decrease their side effects. It was observed that in our results, the IC_{50} of metformin was 385.3 $\mu\text{g/ml}$ in HepG2 cell lines. The metformin inhibits cell viability and induce apoptosis of ovarian, breast colorectal and pancreatic cancers cancer in vitro (20; 21; 22). Metformin inhibits formation of tumor and angiogenesis in mice induced tumor (23).

In the present study; The IC_{50} of cisplatin alone or cisplatin combined with metformin at concentrations 62.5, 125, 250 and 500 $\mu\text{g/ml}$ were 3.39, 3.234, 2.669, 2.402, 1.809 $\mu\text{g/ml}$ in HepG2 cell lines. The IC_{50} of combined metformin showed a significant decreased in cell viability respectively relative to cisplatin alone.

Metformin combined with cisplatin improved the anti-proliferative activity of breast and ovarian cancers as compared with the effect of cisplatin alone (9). It was reported in disagreement of our results that metformin alienates the effect of cisplatin in HepG2 cell line and it has poor effects on MCF-7 and HCT116 (24). Metformin may activate and sensitized cisplatin to

growth of prostatic cancer. The mechanisms of inhibition in positive or negative prostatic cell line with androgen receptor may due to apoptotic pathways (17).

Adjuvant metformin with PTX activate the anticancer of endometrial cancer cell lines through the mTOR pathway. Metformin also potentiates the effects of paclitaxel by inhibiting the cell proliferation of endometrial cancer cell lines via the mTOR pathway (18).

Using metformin as adjuvant therapy with chemotherapeutic drug may increase antiproliferative cancer human cell line and also reduce the cytotoxicity of chemotherapeutic drugs. The aim of this work is studying the effect of the combination of metformin with chemotherapeutic drugs such as cisplatin and paclitaxel on HepG2 cell lines to improve the curative effects and may reduce the adverse effects.

Materials and Methods

The human Hepatic carcinoma cell line HepG2 was purchased from King Fahad Research center. Dulbecco's modified Eagle's medium (DMEM)-high glucose was purchased from UFC Bi tech. Cisplatin, paclitaxel and metformin were obtained from King Faisal Specialist Hospital and Research Center pharmacy. The HepG2 cells were seeded in DMEM supplemented with 10% fetal bovine serum (FBS), 5% penicillin-streptomycin and L-glutamine.

HepG2 cells were incubated at 37°C and 5% CO₂. 0.05% trypsin /EDTA was used to detached monolayer cells for 5 minutes. Suspended cells was counted by using hemocytometer after inactivating trypsin by adding medium. 100 µl of HepG2 cells were seeded to each well in 96 plates containing 10000 cells/well and incubated for 24 hour. After incubation and removing the media, 100 µl of the different concentration of cisplatin (3.75-50 µg/ml), paclitaxel (7.5-120 ng/ml), and metformin (62.5-1000 µg/ml) and combined metformin with cisplatin or paclitaxel were added to 96 plates and incubated 24 and 48 hours. 10 µl methyl thiazolyl tetrazolium assay (MTT) (5 mg/ml) was added and incubated 4 hours to detect cell proliferation and determine IC₅₀. The medium was replaced with 100 µl DMSO; absorbance was read at 570 nm after 20 min. The IC₅₀ of metformin 62.5, 125, 250 and 500 combined with different concentration of cisplatin (3.75-50 µg/ml) or combined with paclitaxel (7.5-120 ng/ml) were determined using HepG2.

Statistical analysis

Data obtained was represented as mean values of % of viability ± SD of 3 independent experiments performed in triplicate. IC₅₀ was determined and figures were drawn using program Graph Pad Prism version 6.

deaths in 2018. 17 % of deaths is due to cancer (1,2).

Cisplatin is a member of a class of platinum containing chemotherapy drugs used to treat a variety of malignancies, including ovarian, lung, gastric and head and neck cancer (3). The predominant anti-cancer mechanism of cisplatin is by binding to DNA, which causes crosslinking of DNA strands and ultimately blocks tumor cell proliferation and induces apoptosis. Metformin has been demonstrated to act synergistically with cisplatin to decrease tumor size and inhibit angiogenesis in mice, indicating that it could be a reasonable candidate for combination with cisplatin-dependent therapy (4).

Paclitaxel (PTX) is natural products and classified as the taxane class of chemotherapeutics. It was extracted from specific bark from the Pacific yew tree. Paclitaxel is accepted as effective anticancer activity in International Cancer Institute Screen of Plants and Natural products. It is used to as chemotherapeutic drugs in patients with solid tumor; carcinoma; sarcoma and melanoma. Paclitaxel was used in patients with breast, uterus, cervixes, small-cell lung and urinary bladder cancers (5,6).

Metformin (1,1-dimethylbiguanide hydrochloride), an oral anti-diabetic drug, is widely used by overweight and obese people with type-2 diabetes. Prior studies have identified metformin as having

potential as an anticancer drug (4,7). Aside from its hypoglycemic effect, metformin it has been recently found capable of reducing the risk of cancer by inhibiting the growth of various tumor cells, including those of breast cancer, ovarian cancer, pancreatic cancer and other malignancies (8,9). The incidence of cancer and rate of mortality increase diabetic patients treated with insulin or drugs activates insulin secretion such as sulfonylureas than that diabetic patients treated with metformin (10, 11).

Metformin is used as adjuvant therapy to treat of ovarian and breast in vitro. It has antitumor activity in human cancer cell lines. Metformin may arrest of cell cycle, induce apoptosis and autophagy of human cancer cell lines (12). The combination of metformin and paclitaxel arrest of cell cycle in the G2-M phase in MCF-7 and A549 cells. This combinations plays a specific roles of the AMPK signaling pathway (13, 14). In vivo study, it was observed that metformin may prevent metastasis and formation of new blood vessels in xenographic rats induced ovarian cancer cell lines. Metformin may reduce the cytotoxicity of cis-platin in human leukemia, glioma and neuroblastoma cell lines by the mechanism of upregulations of the Akt pathway through AMPK signaling pathway (15,16).

The combined bicalutamide drug with metformin may inhibit the

In *vitro* Modulation the efficacy of anticancer drugs by using metformin on cancer human cell lines

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Abstract. the efficacy of anticancer drugs depend on bioavailability and less toxicity. The aim of this study is to using metformin as adjuvant therapy with chemotherapeutic drug to enhance its potential as anti-proliferative cancer cells and also reduce the cytotoxicity of chemotherapeutic drugs. This was carried out by studying the effect of the combination of metformin with cisplatin and paclitaxel on HepG2 cell lines to improve the curative effects and may reduce the adverse effects. HepG2 human cell lines was used to evaluate antitumor activity of cisplatin or paclitaxel (PTX) combined with metformin by using the methyl thiazolyl tetrazolium assay (MTT) to detect anti-proliferative activity and determine IC₅₀ for dose and time dependent assay. The IC₅₀ of different concentration of combination of metformin and paclitaxel or cisplatin were determined. Results obtained showed that, addition of metformin (125, 250 and 500 µg) respectively to cisplatin or paclitaxel reduced IC₅₀ relative to each drug alone in the three cell lines used. The most effect of IC₅₀ obtained from combination of metformin with cisplatin or paclitaxel was observed on HepG2. It was concluded that the combination of metformine and cisplatin or paclitaxel on the hepatic cancer decrease IC₅₀ than that used the chemotherapeutic drugs alone. It is recommended study the efficacy of the combination of therapeutic drug with metformin in animal as promising with high efficacy and less toxicity.

Key words: Metformin; cisplatin; paclitaxel; cancer cell lines

Introduction

Cancer is abnormal cell growth due to genetic mutations of key genes. Key genes are oncogenes, tumor suppressor genes (TSG), DNA repair genes; and apoptotic and apoptotic genes). The etiology of Cancer is

multifactorial due to environmental factors and inherited of key genes. The invading of cancer cells to the different organs in the body cause tumor and deformity of organs leads to death. It was reported 9.6 million