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In-Vitro Antiproliferative Analysis of Metformin Hydrochloride on Androgen-Sensitive, LNCAP and Androgen-Insensitive, PC-3 Human Prostate Cancer Cell Lines

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Abstract

Prostate cancer is one of the diseases worldwide that causes cancer-related deaths in men. Metformin is an antidiabetic drug that has been in use for over two decades for the treatment of Type II Diabetes mellitus (DM2). The purpose of this study was to evaluate the anti-proliferative property of metformin hydrochloride on androgen-sensitive, LNCAP and androgen-insensitive, PC-3 human prostate cancer cell lines at different concentrations (μM and mM) using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Metformin hydrochloride displayed a stronger cytotoxicity on the androgen-insensitive PC-3 than on the androgen-sensitive human prostate cancer cell lines. For both cell lines, the antiproliferative activity of metformin hydrochloride was best displayed at 0.1 mM concentration with average cell death percentage of 60% after 120-hour exposure.

Keywords

Metformin Hydrochloride, Prostate Cancer, Type II Diabetes Mellitus (DM2)

1. Introduction

Prostate cancer is one of the foremost diseases worldwide that causes cancer-related deaths in men. It is the second most common noncutaneous cancer that affects thousands of men each year in the United States and occurs in older men above the age of 65 years. Every year, 20.7 per 100,000 men per year die of prostate cancer and one out of nine men is diagnosed with prostate cancer. It was estimated that 26,120 (4.4%) with prostate cancer will die in 2016 out of 180,890 new cases diagnosed [1]. Type II diabetes mellitus (DM2) is a common disease

that affects about 9.3% (29 million) of people aged 20 years or older in the United States [2] [3]. Metformin, which belongs to the biguanide family, has been used for over two decades as the first line oral therapy for DM2 due to its clinical efficacy and low toxicity profile. The drug suppresses the hepatic glucose production through a mild and transient inhibition of the mitochondrial respiratory chain complex I [4]. This leads to the activation of AMPK (AMP-activated protein kinase). This effect has been suggested that metformin inhibits cancer cell proliferation and induces apoptosis by decreasing the energy disposition due to AMP: ATP ratio elevation and AMPK activation which can suppress tumor formation [5]. Different epidemiology studies have suggested that there is a decrease in the incidence and mortality rate of different cancers with the use of metformin. Case-control studies revealed that new users of metformin have a low risk of developing cancer [6] [7] [8] [9]. In this study, we describe findings supporting the antiproliferative property of metformin hydrochloride on human prostate cancer cell lines, LNCaP and PC-3.

2. Method

In Vitro Antiproliferative Assay

The MTT Cell Proliferation Assay was used in assessing the cytotoxicity profile of metformin hydrochloride. Cell lines, Prostate adenocarcinoma; (Homo sapiens)—PC-3 and LNCaP, purchased from American Type Culture Collection (ATCC) were cultured and maintained in RPMI 1640 medium containing 10% FBS, penicillin-streptomycin, sodium pyruvate, glutamine and non-essential amino acids at 37˚C in 5% CO_2 humidified environment. Cell lines were harvested upon confluency, and then seeded into 96-well plates (with the exception of a two rows designated for a blank and vehicular control). The PC-3 cells were seeded at densities between 0.75×10^4 and 1.5×10^4 cells per well while the LNCaP was seeded at a density of 2.0×10^4. Cells were given 24 hours to attach to the wells. Proceeding the 24-hour incubation period, the RPMI medium was aspirated and replaced with 100 µL of the metformin hydrochloride in either fresh medium or DMSO. Metformin hydrochloride concentrations used were 3 µM, 5 µM, 8 µM, 0.1 mM, 3 mM, 5 mM, and 8 mM. The blank row contained wells of cells within the required density ranges, and the control row contained cells within the density ranges and 100 µL of 0.001% DMSO. Five replicates of each concentration, in addition to the blank and control, were run in parallel. Viability of the cells was determined quantitatively by treatment with the reducing agent 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). A MultiSkan FT spectrophotometer was used in determining the optical densities of the solutions of the resulting formazan products for each assay. The MTT assays were performed after 72-hour and 120-hour exposure of each cell line to each concentration of the drug. 10 µL of MTT reagent was added to each well at the end of the 72-hour and 120-hour incubation periods, and the plate(s) were returned to the humidified environment for 2 to 4 hours or until a purple dye is visible. When the purple precipitate is clearly visible under an inverted micro-

scope, 100 µL of Detergent Reagent was added to all wells. Cell viability was determined by comparing the optical density (OD) of the treated versus the control (Corrected OD of the treated/Corrected OD of control × 100%) [10].

3. Results and Discussion

Antiproliferation activity using MTT assay was used to determine the anticancer activity of metformin hydrochloride, an antidiabetic drug. The results indicated that the activity of metformin hydrochloride uses both dose-and time-dependents cytotoxicity (Figure 1).

The average cell death ranging between 20% and 60% was observed when androgen-dependent, LNCaP, and androgen-independent, PC-3, human prostate cancer cell lines were treated with metformin hydrochloride at different concentrations of 3 µM, 5 µM, 8 µM, 0.1 mM, 3 mM, 5 mM, and 8 mM. In PC-3 cell line, the average cell death percentage was between 21% and 33% at µM concentrations at 72- and 120-hour exposure respectively. The average cell death percentage increases to 53% and 57% after 72- and 120-hour exposure respectively at mM concentrations. In LNCaP cell line, the average cell death percentage did not change (25%) at µM concentrations at both 72- and 120-hr exposure. The same trend was observed at mM concentrations. The average cell death percentage did not change at 72-hour exposure (~47%) and about 49% was observed after 120-hour exposure of the cell line to metformin hydrochloride.

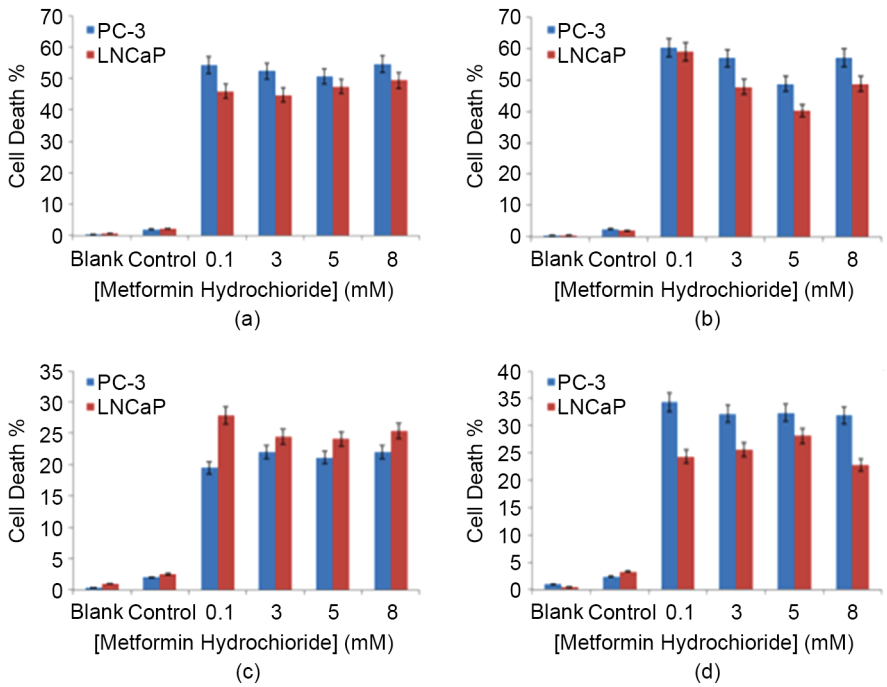

Figure 1. Average cytotoxic effects of metformin hydrochloride on human prostate cancer cell lines. The cancer cell lines were treated with 0.001% DMSO (control). (a) Cell death percentage with metformin at mM concentrations after 72 hours of incubation, (b) metformin at mM concentrations after 120 hours of incubation, (c) metformin at µM concentrations after 72 hours of incubation and (d) metformin at µM concentrations after 120 hours of incubation.

The result of this study showed that metformin hydrochloride displayed cytotoxicity to some extent regardless of whether the prostate cancer cell line is androgen-sensitive or androgen-insensitive after 72 and 120-hour exposure. A stronger effect was observed on the androgen-insensitive (PC-3) than on the androgen-sensitive (LNCaP) prostate cancer cell lines. This finding also indicates that additional experiments using combination studies with chemotherapeutic agents like doxorubicin and cisplatin [11] [12] need to be performed to evaluate the potential strategy to treat prostate cancer patients with DM2. Although, more studies have to be done on mice having prostate cancer and treating them with metformin and chemotherapeutic agents mentioned above. This would be the first step before clinical trials can commence. Therefore, for elderly males diagnosed with diabetes and prostate cancer, metformin may aid in suppressing the cancer growth by serving as a defense mechanism for DM2; thereby reducing the cancer mortality rates in obese men.

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