Journal of Pharmaceutical and Biomedical Analysis 154 (2018) 294–301

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

# Unveiling anticancer potential of glibenclamide: Its synergistic cytotoxicity with doxorubicin on cancer cells

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ARTICLE INFO

*Article history:*
Received 30 November 2017
Received in revised form 12 March 2018
Accepted 13 March 2018
Available online 14 March 2018

*Keywords:*
Glibenclamide
DNA interaction
Grove binding
Combination therapy
Anti-cancer

ABSTRACT

Drug repurposing has been an emerging therapeutic strategy, which involves exploration of a new therapeutic approach for the use of an existing drug. Glibenclamide (Gli) is an anti-diabetic sulfonylurea drug extensively used for the treatment of type-2 diabetes, it has also been shown to possess antiproliferative effect against several types of tumors. The present study was executed to understand the mechanisms underlying the interaction of Gli with DNA under physiological conditions. The binding mechanism of Gli with DNA was scrutinized by UV–vis absorption spectroscopy and fluorescence emission spectroscopy. The conformational changes and electrochemical properties were analyzed by circular dichroism spectroscopy and cyclic voltammetry. Isothermal titration calorimetry was employed to examine the thermodynamic changes and molecular docking technique used to analyze the interaction mode of Gli with DNA. The spectroscopic studies revealed that Gli interacts with DNA through groove binding mode. Further, isothermal titration calorimetry depicted a stronger mode of interaction favorably groove-binding. Recently, systemic combination therapy has shown significant promise in inhibiting multiple targets simultaneously yielding high therapeutic competence with lesser side effects. With this concern, we intended to study the combined cytotoxicity of Gli with doxorubicin (Dox). The results of MTT assay and acridine orange (AO)/ethidium bromide (EtBr) staining showed synergistic cytotoxicity of Gli + Dox combination on HepG2 & A549 cells. The present study documents the intricate mechanism of Gli–DNA interaction and delivers a multifaceted access for chemotherapy by Gli + Dox combination.



## 1. Introduction

Cancer is a disease characterized by uncontrolled cell growth and most common life threatening condition which leads to increased morbidity and mortality worldwide. Among various cancers, hepatocellular carcinoma (HCC) is the 5th leading cause of the cancer related mortality worldwide. HCC is undetectable and asymptomatic at the earlier stages and it commonly occurs in patients with chronic liver diseases [1]. Similarly, lung cancer is the leading cause of cancer death in both men and women [2]. There are several traditional approaches for the cancer treatment such as chemotherapy, radiotherapy, immunotherapy and surgical removal of cancer. Among them, chemotherapy is the most commonly practiced cancer treatment [3]. Further, most of the traditional cancer therapies have been classified as mono therapy which use agents targeting similar pathway such as DNA replication to target the proliferating cells. Even-though the chemotherapy possesses considerable efficacy against various cancer types, however it also has the limitation due to the systemic toxicity by non-specific destruction of both cancer and normal cells especially highly proliferating cells and the resistance developed by the cancerous cell through adaptation of new salvage pathways [4,5,6]. In this conception, currently the researchers intend to use combination of agents to treat the cancer cells. The combination therapy is an effective strategy for the various challenging cancer treatments than mono therapy since it functions through multiple targets [7].

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https://doi.org/10.1016/j.jpba.2018.03.025
0731-7085/

In recent years, new strategy for cancer therapy is drug repositioning in which the drug utilized for noncancerous diseases are being recognized for their anticancer potential. This strategy is effective since it uses an FDA approved drug with a known pharmacokinetic profile [8]. The combination therapy is an effectual treatment when the combined agents target the cancer cell through multiple pathways. The survival capacity and reproducibility of the cell is completely dependent upon its highly regulated DNA replication and transcription, necessary for synthesis of proteins and execution of cellular functions. The cancer cells alter the normal expression pattern of DNA to favor their survival and uncontrolled replication [9]. In cancer treatments, several targets are being employed to suppress or destroy the cancerous cell. Since DNA repair pathways are essential for the survival and cell proliferation in various cancers, researchers have shown interest to target DNA repair pathways [10].

Natural or synthetic small molecules could be effective in targeting DNA and could lead to the suppression of gene expression and subsequent cell death [11]. The interactions of small molecules with DNA are of two types namely, covalent and non-covalent interactions. In stronger covalent mode of interaction small molecules bind with DNA strands through alkylation and base pair cross linking, whereas in weaker non-covalent mode, small molecules bind with DNA through intercalation and groove binding [12]. From this conception, in the present study, we set out to investigate the mode of interaction of anti-diabetic drug glibenclamide (Gli) ($C_{23}H_{28}ClN_3O_5S$) on DNA and its anti-cancer potential against liver and lung cancer cells to validate the new combinatorial therapy. Gli is a second generation sulfonylurea drug, which stimulates insulin production in pancreatic beta cells by raising cytoplasmic calcium level [13]. Gli also has been reported to have anticancer property in various types of cancer [14]. However, there is no report on the interaction mode of Gli with DNA and its synergistic anticancer effects with doxorubicin (Dox). In the current study, the mode of interaction of Gli with calf thymus DNA was assessed by various physiochemical and bioinformatics approach. The well-known anticancer compound doxorubicin(Dox) targets the cell DNA, inhibits the cell proliferation and induces apoptosis [15]. Further, in the current work the combined anticancer potential of Gli and Dox were studied on liver and lung cancer cells.

## 2. Materials and methods

### 2.1. Materials

Calf thymus DNA (CT-DNA) sodium salt and Gli were purchased from Sigma Aldrich chemicals (Sigma Aldrich, USA). Hoechst 3328 and EtBr were purchased from Himedia, India. Other chemicals utilized in this study were of analytical grade.

### 2.2. Stock solution preparation

CT-DNA was dissolved in 10 mM Tris-HCl buffer (pH 7.4) at a concentration of 10 mg/ml and stirred gently for 24 h to get a homogenous solution then stored at 4 °C for further use. The concentration of CT-DNA solution was estimated at 260 nm using molar extinction coefficient $\varepsilon_{260}$ such as 6600 $cm^{-1}M^{-1}$. The 260/280 nm ratio of the CT-DNA was verified as 1.8 which indicates the purity of CT-DNA i.e free of protein and RNA contamination. The final concentration of CT-DNA was 19.8 mM. The final working CT-DNA solution was prepared at a concentration of 100 mM. The stock solution of Gli was prepared at a concentration of 5 mM with absolute ethanol and 1 mM stock was prepared from 5 mM stock by diluting with Tris-HCl buffer. Various concentrations of working Gli (5–100 μM) were prepared from 1 and 5 mM stock using Tris-HCl buffer.

### 2.3. Methods

#### 2.3.1. UV–vis spectroscopic analysis

The spectral properties of CT-DNA alone and CT-DNA with various concentrations of Gli-DNA complex were analyzed with Shimadzu UV–vis spectrophotometer, (model-UV-1800) with a slit of 2 nm at 25 °C, with 1 cm path length quartz cuvettes. The linear double reciprocal plot was illustrated for 1/A-$A_0$ (A- absorbance of Gli- DNA complex; $A_0$–absorbance of DNA alone) against 1/C (C- concentration of Gli) and the binding constant was determined from the ratio of the intercept to slope [16].

#### 2.3.2. Fluorescence emission spectroscopy study

Competitive fluorescence displacement assay was performed with SPECTRA-MAX M5 multi-mode reader. Different concentrations of Gli (5–100 mM) were mixed with 100 μM of CT-DNA in the presence of EtBr – an intercalating dye (2 μM). EtBr displacement was assessed with the fluorescence emission recordings of 560–640 nm with the excitation at 520 nm at 25 °C. Similarly, the fluorescence displacement to determine groove binding was evaluated by increasing concentrations of Gli (5–100 mM) in the mixture of constant concentration of CT-DNA (100 μM) and Hoechst 3328 (5 μM) with the emission scan of 450–600 nm. The quenching constant ($K_{SV}$) was determined by following Stern-Volmer equation for Gli-DNA complex [16].

$$\frac{F_0}{F} = 1 + K_{SV}[Q]$$

Where $F_0$ and F are the fluorescence intensities in the absence and presence of quencher (Gli) and Q is the concentration of quencher. $K_{SV}$ is the Stern-Volmer quenching constant which can be deduced from the linear relationship between $\frac{F_0}{F}$ and [Q].

#### 2.3.3. Circular dichroism (CD) spectroscopy study

CD spectra of CT-DNA and Gli-DNA complex were executed using JASCO J-815 CD spectrophotometer with the baseline correction using Tris-HCl at pH7.4 in a 1 mm path length quartz cuvette. In this, different concentrations of Gli (5–100 μM) were mixed with a fixed concentration of DNA (100 μM) and their CD spectra were recorded at wavelength range of 200–320 nm with a scan speed of 50 nm/min at 25 °C [16].

#### 2.3.4. Cyclic voltammetric measurement

The electrochemical property of Gli and DNA complex were figured out by mixing different concentrations of Gli (5–100 μM) and 100 μM DNA in 50 mL voltammetric cell and 10 mM Tris-HCl as a supporting electrolyte in autolab type (PGSTAT 302 N) electrochemical analyzer. The analyzer consists of three electrode systems namely, a working electrode comprised of carbon glass, reference electrode made of Ag/AgCl and a counter electrode made of platinum wire (Pt). The samples were read at a scan rate of 100 mV/s. The binding constant of Gli-DNA complex was determined from the intercept and slop of the plot such as 1/Q vs1/(1-$i/i_0$) where, $i$ is the peak current of Gli-DNA complex, $i_0$ is the peak current of DNA alone and Q is the different concentrations of Gli [16].

#### 2.3.5. Isothermal titration calorimetric investigation

The interaction of Gli and CT-DNA was further confirmed with isothermal titration calorimetry (ITC) technique using NANO ITC. All the buffers and samples for ITC analysis were prepared with degassed buffer and the temperature was maintained at 25 °C. The stirring speed was adjusted to 150 rpm. The study was initiated with baseline correction using degassed Tris-buffer (10 mM pH7.4),