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Antiproliferative activities of procainamide and its binding with calf thymus DNA through multi-spectroscopic and computational approaches

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ABSTRACT

The interaction between procainamide with ct-DNA was seen through several experimental and theoretical methods. The fluorescence intensity of procainamide decreased on increasing the concentration of ct-DNA and the quenching process was found to be static with approximately 1:1 binding between ct-DNA and procainamide. UV absorption spectroscopy gave an idea about minor groove binding which was further confirmed by the dye displacement method of DAPI/EtBr bounded ct-DNA interaction. Minor groove binding was also evidenced from the collective information obtained from DNA melting, viscosity and CD spectroscopy. Molecular docking simulations presented that procainamide bound in the minor groove (AT rich) region of B-DNA structures. From thermodynamic point of view the binding interaction between procainamide and ct-DNA was spontaneous process with liberation of energy and overall ordering of system. Hydrogen bonding was found to play important role as suggested by the values of thermodynamic parameters. Whereas from molecular docking simulations it was exposed that hydrogen bonding and hydrophobic interactions were crucial in the binding of ct-DNA and procainamide. DFT method was also used to calculate the Frontier molecular orbitals (HOMO and LUMO) of procainamide which were further used to calculate chemical potential (μ), chemical hardness (η) and fraction number of electrons (ΔN) from procainamide to DNA. Procainamide was found to act as electron donor to DNA bases excpet guanine. Finally, elucidation of anticancer activity revealed that procainamide possesses potential cytotoxicity against MCF-7 breast cancer cells and able to induce significant level of apoptosis at concentrations below IC_{50} value.

1. Introduction

Procainamide is a tertiary amine compound and served as a cardiac depressant, particularly as an antiarrhythmic medicine used to treat cardiac arrhythmic taking place in patients with heart problems. It was developed to upsurge the actual refractory period of the atria and ventricles of the heart [1–4]. Procainamide is quickly absorbed in the body after intravenous or oral administration [5]. It is also associated with several side effects such as, anorexia, vomiting, nausea and diarrhea, fever, joint and muscle pain, etc. [6]. Toxicity of procainamide towards the lungs has also been reported in which it causes the pulmonary fibrosis [7].

Though, it was developed as type IA antiarrhythmic drug, several anticancer activities of procainamide have also been observed [8]. Procainamide was found to protect mice against toxic doses of cisplatin and greatly decreased the weight loss brought by cisplatin. Moreover, it prevented the increased plasma urea nitrogen levels caused by administration of cisplatin as well as the tubular degenerative changes [9]. Procainamide also found to enhance the efficacy of cisplatin in P388 leukemic mice changes [9] as well as increase the tamoxifen response in ERα-positive and ERβ-negative breast cancers [10]. Recently, it was found to improve the anticancer activities of cisplatin against in murine P388, human A2780 and A549 cells [11] and the toxicity of liposome-cisplatin combination was also increased in its presence [12].

Procainamide is also an efficient molecule to design the stable radiolabeling substance for heart imaging [13]. Furthermore, due to its specific binding tendency with melanin pigment, the novel radiotracer was designed for *in vivo* imaging of melanoma tumors [14]. The improvement in the anticholitic activity of 5-aminosalicylic acid was observed when it was conjugated with procainamide via an azo bond [15].

The most interesting role of procainamide as anticancer agent is to promote the demethylation of DNA which is a very important hallmark in cancer related research [16]. DNA demethylation is a practice in which inhibitor distresses the activity of DNA methyltransferases. Procainamide induced demethylation and able to reactivation of the tumor

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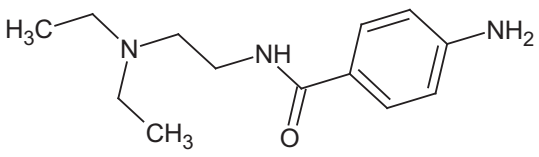
suppressing genes ER, RARβ3, and p16 in cultured cells [17]. Lee et al. found that procainamide unambiguously prevents the hemimethylase activity of DNA methyltransferase 1 (DNMT1), which is believed to be accountable for maintaining DNA methylation patterns during replication though it was not an effective suppressor of the de novo methyltransferases DNMT3a and DNMT3b2 [18]. Secreted Wingless type (Wnt) ligands are found to be participated in tumor developmental processes and oncogenesis. Aberrant promoter methylation of Wnt inhibitory factor-1 (WIF-1) is a fundamental mechanism of epigenetic silencing in human cancers. Procaine and procainamide were found to reactivate WIF-1 in these cancer cells and downregulate the Wnt canonical pathway subsequently can prevent the development of lung cancer [19].

Since applications of procainamide as anticancer agent are of importance in cancer therapy, study on its interaction and binding mechanism with DNA is of particular interests. DNA is a vital genomic component of life that transports maximum of the hereditary information and permits the biological synthesis of proteins and enzymes through the replication and transcription [20]. Interaction of DNA with small molecules or ligands takes place via various modes for instance (i) intercalation of the small molecule inside the DNA base pairs that causes the distortion in the DNA double helix, (ii) electrostatic interaction between negatively charged phosphate backbone of DNA and positive ends of small molecules, ligands or drugs, (iii) groove binding which involves either minor groove binding preferably for small molecules or major groove binding for large molecules such as polypeptides. Groove binding doesn't influence the double helix of DNA too much unlike intercalation as both are correlated to the grooves in DNA double helix, whereas electrostatic binding takes place outside the groove [21,22].

The binding mode of a drug, in general, and an anticancer drug, in particular, with DNA are gaining attention to evaluate the mechanism of action of the drugs [23–25] because DNA is the prime target of several drug molecules for attenuation the transcription and replication. Understanding the binding mechanism of drug and DNA can be helpful to design the new pharmaceutical agents for targeting DNA [26,27]. Therefore, in the present study we have investigated the mode of binding of procainamide to the ct-DNA experimentally, using a bunch of techniques, as well as computationally using molecular docking and DFT calculations. Furthermore, cytotoxic activities of procainamide on human breast cancer (MCF-7) cells have also been performed. The structure of procainamide is given in Scheme 1.

2. Experimental

UV–visible studies as well as DNA melting studies were performed on Perkin-Elmer Lambda 45 Spectrophotometer using quartz cuvettes of 1 cm path length. Fluorescence spectroscopy was executed on Hitachi F-7000 spectrfluorometer and for quenching experiments the excitation wavelength was 290 nm and the excitation and emission slits were fixed at 10 nm with PMT voltage of 400 V. In case of competitive displacement assays using DAPI and EtBr, the excitation wavelengths were kept as 341 nm and 480 nm, respectively. CD spectroscopy was done on Jasco J-815 spectropolarimeter using 2 mm quartz cell. Ostwald viscometer was used to calculate the relative viscosities of ct-DNA and ct-DNA–procainamide complex. The geometries of procainaimde and DNA bases were optimized at DFT/B3LYP/def2SVP/J [28–30] by ORCA



Scheme 1. Structural formula of procainamide.

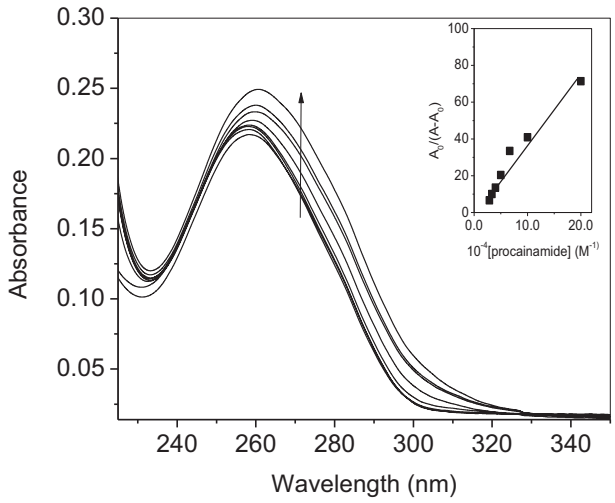


Fig. 1. Difference absorption spectra of ct-DNA (30×10^{-6} M L^{-1}) in presence of increasing amount of procainamide (0, 5, 10, 15, 20, 25, 30, 35×10^{-6} M L^{-1}) at 25 °C. Inset: Benesi Hildebrand plot.

software [31–33]. Autodock 4.2.3 Program was used to perform docking calculations of DNA with procainamide [34].

The MCF-7 human breast adenocarcinoma cell line was obtained from American Type Culture Collection (ATCC, Rockville, MD, USA) and cells were further grown in the lab using standard protocols. A CellTitre 96® non-radioactive cell proliferations assay kit (Promega, Madison, WI, USA) was used to analyze the cytotoxic activity of procainamide through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay. Morphological changes in the cells were observed under a phase contrast inverted microscope equipped with a digital camera (Olympus IX51, Tokyo, Japan) at 100× magnification after growing the cells in presence of various amounts of procainamide. Apoptotic morphological changes were seen by the AO-EtBr dual staining method. The incidence of apoptosis was measured in MCF-7 cells by flow cytometry using the annexin V-FITC and propidium Iodide (PI) apoptosis detection kit (BD Biosciences, San Diego, USA).

The detailed experimental procedure is given in ESI.

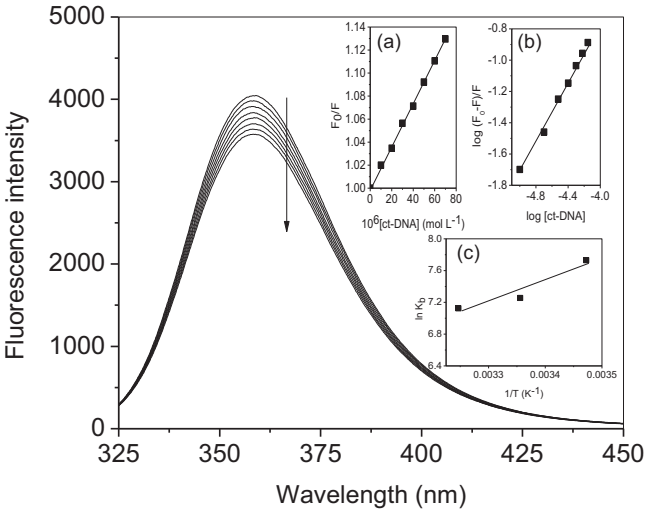


Fig. 2. Fluorescence emission spectra of procainamide (30×10^{-6} M L^{-1}) in the presence of increasing amount of ct-DNA (5, 10, 15, 20, 25, 30×10^{-6} M L^{-1}) at 25 °C. Inset: (a) Stern-Volmer plots of ct-DNA interaction with procainamide. (b) Plot of log $(F_0 - F)/F$ as a function of log [ct-DNA]. (c) van 't Hoff plot of ct-DNA-procainamide interaction.