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FTIR spectral signature of the effect of cardiotonic steroids with antitumoral properties on a prostate cancer cell line

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ABSTRACT

We show in the present work that the infrared (IR) spectrum of human PC-3 prostate cancer cells exposed to anticancer drugs could offer a unique opportunity to get a fingerprint of all the major biochemical components (DNA, RNA, proteins, lipids, etc.) present in the cells and to identify with high sensitivity the signature of the metabolic changes induced by anticancer drugs.
We investigated here the FTIR-related signatures of the effect of 4 structurally-related cardiotonic steroids (CS), i.e. ouabain, 19-hydroxy-2″-oxovoruscharin, hellebrin and 19-hydroxy-hellebrin on PC-3 cancer cells incubated between 0 and 36 h in the absence (control) or the presence of the CS. For each molecule a single spectral signature described the largest part of the time dependent modifications with a possible very minor second component. The spectral signatures characterizing the effects of each of the four CS were unique but very similar when compared to the signature of the effect of an intercalating anticancer drug, i.e. doxorubicin, selected as a positive reference compound in our study, suggesting a fully distinct set of cellular perturbations. The current study thus illustrates that Fourier Transform Infrared (FTIR) analyses can be used to identify, among the perturbations induced on a given cancer cell line, the features common to a group of anticancer compounds as well as features specific to every single drug.

1. Introduction

A growing number of potential anticancer agents fails in the course of drug development process suggesting that the selection procedure for progression of molecules from early research stages through pre-clinical trials to the clinic requires significant improvement [1]. High throughput screening procedures remain essentially based either on the ability of new drugs to induce cell death over a panel of several cell lines or on their interaction with key parts of one or several biological pathways [1,2]. A technique able to provide a global fingerprint of drug mechanism of action on cancer cells could be of great interest in the development of therapeutic medicines with new modes of action. We suggest in the present study that the infrared (IR) spectrum of tumor cells exposed to anticancer drugs could offer a unique opportunity to get a signature of all the major biochemical components present in the cells and to characterize, with a high sensitivity, specific metabolic changes induced by new potential anticancer drugs.

Cardiotonic steroids (CS) are well known inotropic drugs for the treatment of congestive heart failure and atrial arrhythmia, and the mechanism of their positive inotropic effect is well characterized [3–6]. Chemically, CS are compounds presenting a steroid nucleus with a lactone moiety at position 17 [3,4]. Glycosylated CS contain a sugar moiety at position 3 [3,4]. The nature of the lactone ring at position 17 defines the class of CS: the cardenolides (with an unsaturated butyrolactone ring) and the bufadienolides (with a α-pyrone ring) [3,4]. Recent studies emphasized potent in vitro and in vivo antitumoral effects of these molecules (reviewed in [3–5]). The Na^+/K^+-ATPase, the so-called sodium pump, is an integral transmembrane protein found in all higher eukaryotic cells that uses energy from ATP hydrolysis to maintain a high K^+ and low Na^+ concentration in the cytoplasm [7]. Recently a non pumping pool of Na^+/K^+-ATPase had been evidenced and localised in caveolae [8], a raft like structure [9]. This pool of Na^+/K^+-ATPase was shown to be associated with different signalling proteins and act as a signal transducer [10–12]. The sodium pump mediates CS-induced effects in a compound, concentration and cell type-specific manner [3,4,13,14]. Exciting recent findings have suggested additional signaling modes of action of the sodium pump, implicating CS in the regulation of several important

Abbreviations: IR, infrared; FTIR, Fourier Transform infrared; CS, cardiotonic steroid; PCA, Principal Component Analysis; PC, Principal Component; ESS, Error sum of square

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cellular processes and highlighting potential new therapeutic roles for these compounds in various diseases, including cancer [3–6]. The increased susceptibility of cancer cells to these compounds supports their potential use as anticancer therapies, and the first generation of glycoside-based anticancer drugs are currently in clinical trials [3–6]. In addition, we have previously demonstrated that cardenolides are able to overcome MDR phenotype in cancer cells [15] as well as the intrinsic resistance of various types of cancers to apoptosis [16,17]. We have demonstrated that cardenolides exert in fact their anticancer activity through the targeting of the alpha subunits of the sodium pump [18] and that the alpha-1 subunit of the sodium pump is overexpressed in a large proportion of non-small-cell lung cancers [18], glioblastoma [16] and melanoma [19]. Because of their closely related structure and action, the CS family is particularly challenging when distinct modes of action are to be identified.

FTIR spectroscopy was used successfully for decades as label free technique for the study of cells [20,21] as well as tissues [22–24]. Absence of reagent remains one of the main advantages of IR spectroscopy among other exploratory techniques such as fluorescence or microarrays. It senses every metabolite of the cells with a great sensitivity without disturbing its integrity and can therefore provide an accurate fingerprint of modifications occurring within the cells. In turn, IR spectroscopy can be used for bacteria or yeast typing at strain level [20,25,26]. With human samples, IR spectroscopy allowed rapid distinction between tumor and normal tissues [27–29] or powerful discrimination between cancer cell lines [30]. For example, our group established IR markers for determination of the *in vivo* aggressiveness level of glioma cell lines [30]. Recently, we evidenced that metabolic perturbations induced by ouabain on the human PC-3 prostate cancer cell line at nM concentration can be monitored by infrared spectroscopy [31]. In the present study, we analyzed spectra of this human PC-3 prostate cancer cell line treated with four different CS during 5 different periods of time. Two of these CS are cardenolides and the remaining two bufadienolides. Each pair of cardenolides and bufadienolides included one natural and one hemisynthetic compound reduced in position 19 of the steroid nucleus (see Fig. 1). As indicated above, CS share a common cellular receptor, the Na^+/K^+-ATPase, but their mechanisms of anticancer action might vary markedly from one compound to another [4,16,32]. In addition, these signatures were compared to the ones induced by doxorubicin, an intercalating agent that is widely used to combat various types of cancers [33] in order to evaluate spectral changes induced by a molecule belonging to a different family.

2. Materials and methods

2.1. Compounds

Doxorubicin and hellebrin were purchased from Sigma-Aldrich S.A. (Bornem, Belgium). Ouabain was purchased from Acros Organics (Geel, Belgium). 19-hydroxy-2″-oxovoruscharin was derived from 2″-oxovoruscharin, a cardenolide identified in the African plant *Calotropis procera*, as detailed elsewhere [34]. In the same manner, 19-hydroxy-hellebrin has been derived from hellebrin as detailed elsewhere [35]. The structure of the CS's is presented in Fig. 1.

2.2. Cell culture

The human prostate cancer PC-3 (CRL-1435) cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA) and was maintained according to the supplier's instructions. Briefly, the cells were incubated at 37 °C in sealed (airtight) Falcon plastic dishes (Nunc, Invitrogen SA, Merelbeke, Belgium) in a humidified atmosphere of 5% CO_2. The cells were kept in exponential growth in RPMI medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (an antibiotic/antimycotic solution), and 1% kanamycin to prevent mycoplasms. Medium and FBS were purchased from Gibco (Invitrogen, Merelbeke, Belgium). Penicillin/strepromycin and kanamycin solutions came from Sigma-Aldrich SA, (Bornem, Belgium). Drug concentrations used throughout this paper were the IC_{50}, defined as the drug concentration required for decreasing the cell population by half after 72 h of growth [34]. The medium was replaced 24 h before delivering the drug to prevent any stress induced by this handling.

For FTIR spectroscopy, cells were suspended after a five-minute treatment with trypsin (0.5 g/L)/EDTA (0.2 g/L) buffer (Gibco, Invitrogen SA, Merelbeke, Belgium). The reaction was stopped by adding 1 ml of culture medium. The cells were pelleted by a 2-minute

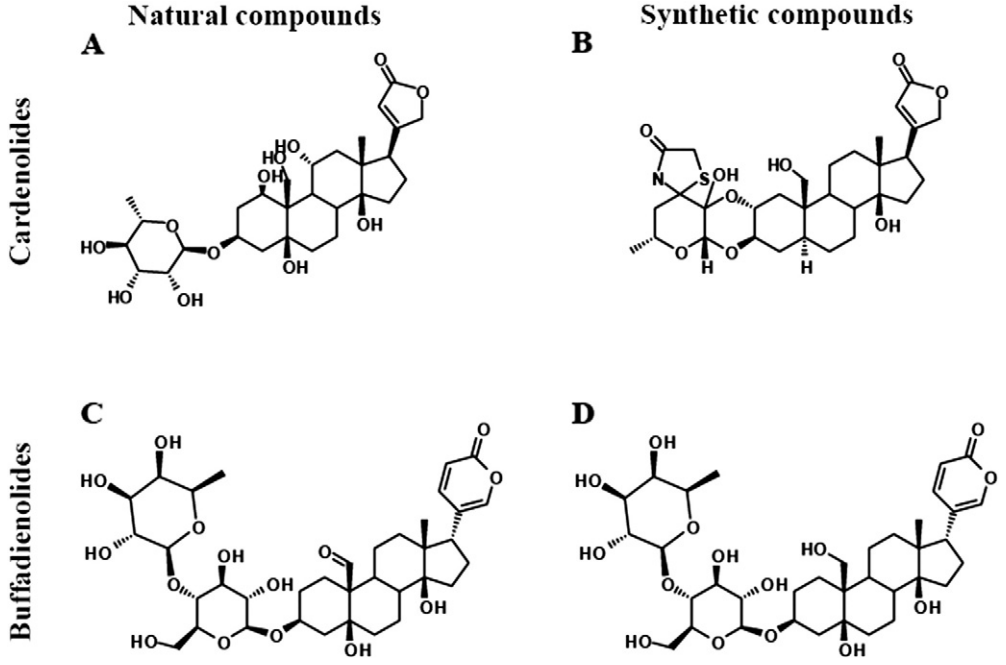

Fig. 1. Structure of cardiotonic steroids used in this study. A: Ouabain, B: 19-hydroxy-2″-oxovoruscharin, C: Hellebrin, D: 19-hydroxy-hellebrin. A and C are natural products. B and D are chemically modified molecules by hydroxylation of respectively A and B at position 19.