Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 138 (2015) 321–330

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Cellular injury evidenced by impedance technology and infrared microspectroscopy

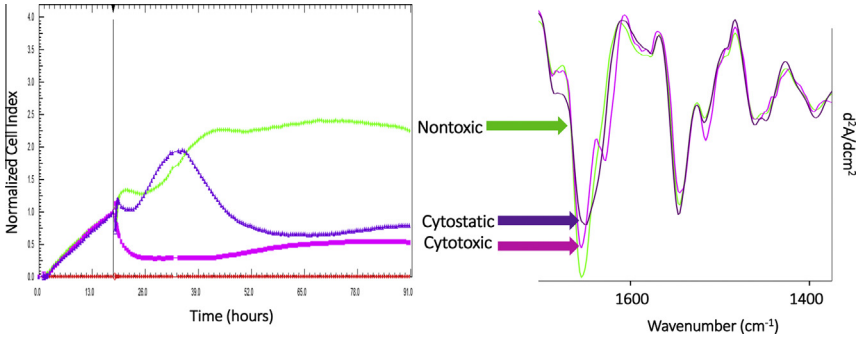
K. le Roux [a], L.C. Prinsloo [b], D. Meyer [a,*]

[a] Department of Biochemistry, University of Pretoria, Pretoria 0002, South Africa
[b] Department of Physics, University of Pretoria, Pretoria 0002, South Africa

HIGHLIGHTS

- FTIR detects different types of cell injury in a manner comparable to RT-CES.
- Spectral differences distinguish cytostasis from cytotoxicity.
- FTIR microspectroscopy detects the reversibility of cytostasis.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 11 June 2014
Received in revised form 20 November 2014
Accepted 21 November 2014
Available online 29 November 2014

Keywords:
FTIR
RT-CES
Cytostatic
Cytotoxic
Cancer

ABSTRACT

Fourier Transform Infrared (FTIR) spectroscopy is finding increasing biological application, for example in the analysis of diseased tissues and cells, cell cycle studies and investigating the mechanisms of action of anticancer drugs. Cancer treatment studies routinely define the types of cell-drug responses as either total cell destruction by the drug (all cells die), moderate damage (cell deterioration where some cells survive) or reversible cell cycle arrest (cytostasis). In this study the loss of viability and related chemical stress experienced by cells treated with the medicinal plant, *Plectranthus ciliatus*, was investigated using real time cell electronic sensing (RT-CES) technology and FTIR microspectroscopy. The use of plants as medicines is well established and ethnobotany has proven that crude extracts can serve as treatments against various ailments. The aim of this study was to determine whether FTIR microspectroscopy would successfully distinguish between different types of cellular injury induced by a potentially anticancerous plant extract. Cervical adenocarcinoma (HeLa) cells were treated with a crude extract of *P ciliatus* and cells monitored using RT-CES to characterize the type of cellular responses induced. Cell populations were then investigated using FTIR microspectroscopy and statistically analysed using One-way Analysis of Variance (ANOVA) and Principal Component Analysis (PCA). The plant extract and a cancer drug control (actinomycin D) induced concentration dependent cellular responses ranging from nontoxic, cytostatic or cytotoxic. Thirteen spectral peaks (915 cm^{-1}, 933 cm^{-1}, 989 cm^{-1}, 1192 cm^{-1}, 1369 cm^{-1}, 1437 cm^{-1}, 1450 cm^{-1}, 1546 cm^{-1}, 1634 cm^{-1}, 1679 cm^{-1} 1772 cm^{-1}, 2874 cm^{-1} and 2962 cm^{-1}) associated with cytotoxicity were significantly (p value < 0.05, one way ANOVA, Tukey test, Bonferroni) altered, while two of the bands were also indicative of early stress related responses. In PCA, poor separation between nontoxic and cytostatic responses was evident while clear separation was linked to cytotoxicity. RT-CES detected morphological changes as indicators of cell injury and could distinguish between viable, cytostatic and cytotoxic responses. FTIR microspectroscopy confirmed that cytostatic

* Corresponding author. Tel.: +27 12 420 3200.
E-mail address: debra.meyer@up.ac.za (D. Meyer).

http://dx.doi.org/10.1016/j.saa.2014.11.089
1386-1425/

cells were viable and could still recover while also describing early cellular stress related responses on a molecular level.

Introduction

Fourier Transform Infrared spectroscopy is a label free, non-destructive, high throughput method which is growing in popularity as the applications increase. The applications of the technique include structure elucidation of compounds [1] and peptides [2,3], identification of different bacterial strains [4,5] and antimicrobial compounds affecting bacterial viability [6], the discrimination of diseased and healthy tissues or cells [7–9], cell cycle analysis [10], the effects of known toxins on cells [11] and the identification of molecular targets of natural and synthetic anticancer drugs [12–15].

Anticancer drugs under development are generally tested *in vitro* to determine whether they can be classified as non-toxic (no or limited cell death caused), cytostatic (arrest of cell proliferation but the cells are essentially still alive) or cytotoxic (considerable cell death induced by the treatment). Cytostatic responses are usually employed to stop uncontrolled tumour growth while cytotoxic responses lead to cell death caused by apoptosis, necrosis or cell lysis [16]. Cytostatic and cytotoxic behaviour plays an important role in the progression of a drug into preclinical and clinical studies. Cytostatic drugs have been found to be metastases preventative and increase the survival rates of patients by delaying the time to disease progression [17]. Cytotoxic drugs, on the other hand, are less selective and the induced cell death could be more detrimental to the patient in terms of side effects. Rixe and Fojo found that a drug elicited either cytostatic or cytotoxic cellular responses depending on its active concentration [17].

Classifications of cytostasis/cytotoxicity are usually decided following spectrophotometric assessment of cell viability in the presence of the drug and a labelling agent, but the use of spectroscopy in the absence of labels is gaining momentum. Conventionally, biochemical labels such as trypan blue, tetrazolium salts and fluorochromes are used to measure a single parameter such as; membrane integrity, enzymatic reactions and intra- or extracellular markers. The major disadvantage of labels is that the experiment becomes an end point analysis measuring only one or a few parameters at a particular time point after which the cells are destroyed. Biochemical labels are also very expensive and can sometimes crossreact with other molecules. Accordingly, new label free methodologies that are less time-consuming and more comprehensive in evaluating multiple cellular components should be developed.

One of the popular label free assays currently in use is RT-CES. The principle of the instrument is based on the growth of adherent cells on gold electrodes. Impedance changes associated with the cells behaviour is measured continuously. When cells initially attach to the electrodes the impedance (electrical resistance) increases, causing an increase in the unit less Cell Index (CI). The CI will increase as the resistance increases, which are due to the cells proliferating or spreading out within the culture well. When a toxin is added to the wells, the cellular response can be measured continuously. Thus, any change in cell size and shape is monitored by changes within the CI. Generally a decrease in CI values could be due to cell volume decreasing, cells rounding up during the activation of cell death or the detachment of cells which leads to a decrease in resistance measured by the electrodes [18]. This technique successfully characterized the type of cellular responses of importance in our research group [19,20] as well as others [16,21–23].

In a previous study, we found that Raman micro-spectroscopy could be used to pre-screen a plant extract for its anticancer activity against the proliferation of leukemic U-937 cells [24]. Since FTIR spectroscopy is complementary to Raman spectroscopy and Raman spectroscopy only interrogates one cell at a time, we set out to determine whether FTIR could distinguish viable from cytostatic and cytotoxic responses in a given cell population following exposure to a cell death inducer. Many publications focus on the cytotoxic drugs and plant extracts or compounds affecting FTIR spectra of treated cells [11,13,14,25,26] while little research had been done on cytostatic responses [15].

In the evaluation of FTIR spectroscopy as a tool for the screening of anticancer drug responses, a cell death inducer is required. In this case a South African traditional medicinal plant was selected. Medicinal plants have long been studied for their effectiveness in treating cancer. Crude extracts of medicinal plants are more potent cytotoxins because of the synergy of the many phytochemicals found in the mixture [24]. The cell death inducer in this investigation was a leaves extract of *Plectranthus ciliatus*. In traditional medicine, plants from this genus are used to treat ailments of the digestive tract, respiratory system, nervous system, inflammation, infections, pain and different types of cancers [27]. The plant's anticancer activity was previously demonstrated by our group [28]. The aim of this study was to determine whether FTIR microspectroscopy could successfully distinguish between different types of cellular injury induced by a potentially anticancerous plant extract.

Methods

Plant material and extraction

P. ciliatus leaves were harvested in the Manie van der Schijff botanical garden, Pretoria, South Africa. The leaves were authenticated and a voucher specimen deposited in the Schweickerdt Herbarium, Pretoria, South Africa. The crude extract of the leaves was prepared and kindly donated by Dr. T.P. Kapewangolo, Department of Biochemistry, University of Pretoria. The plant extraction procedure was done as previously described [20]. The crude extract was stored at 4 °C until use. Stock solutions were freshly prepared before each experiment by dissolving the plant extract in cell culture tested dimethyl sulfoxide (DMSO) (Sigma, Germany).

Cell culture

Human cervix adenocarcinoma (HeLa) cells were purchased from Highveld Biological (Pty) Ltd. (Johannesburg, South Africa) and grown in standard culture flasks in a humidified incubator at 37 °C and 5% CO_2. The growth media was Minimum Essential Medium (MEM), supplemented with 5% heat-inactivated foetal bovine serum (FBS) (Hyclone, Separations, Johannesburg, South Africa) and antimicrobial cocktail (100 U/mL penicillin, 100 μg/mL streptomycin and 250 μg/L fungizone) (Hyclone, Separations, Johannesburg, South Africa). The cells were maintained by sub-culturing the cells when 80-90% confluence was reached.