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Review

FTIR spectral signature of anticancer drugs. Can drug mode of action be identified?☆

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ABSTRACT

Infrared spectroscopy has brought invaluable information about proteins and about the mechanism of action of enzymes. These achievements are difficult to transpose to living organisms as all biological molecules absorb in the mid infrared, with usually a high degree of overlap. Deciphering the contribution of each enzyme is therefore almost impossible. On the other hand, small changes in the infrared spectra of cells induced by environmental conditions or drugs may provide an accurate signature of the metabolic shift experienced by the cell as a response to a change in the growth medium.

The present paper aims at reviewing the contribution of infrared spectroscopy to the description of small chemical changes that occur in cells when they are exposed to a drug. In particular, this review will focus on cancer cells and anti-cancer drugs. Results accumulated so far tend to demonstrate that infrared spectroscopy could be a very accurate descriptor of the mode of action of anticancer drugs. If confirmed, such a segmentation of potential drugs according to their "mode of action" will be invaluable for the discovery of new therapeutic molecules. This article is part of a Special Issue entitled: Physiological Enzymology and Protein Functions.

1. Introduction

Infrared spectroscopy has brought invaluable information about the mechanism of action of enzymes. To quote just an example among many, the different steps of the catalytic cycle of P-type ATPases have been thoroughly characterized at atomic/molecular level since the nineties in the Ca^{++}-ATPase [1–10] as well as in various H^+ and H^+,K^+-ATPase [11–22]. When reaction can be triggered by light [23–35], fast kinetics have been obtained, describing time resolved reactions in very high detail [24,36–66].

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The present paper aims at reviewing the contribution of infrared spectroscopy to the description of small chemical changes that occur in cells when they are exposed to a drug. In particular, this review will focus on cancer cells and anti-cancer drugs. Results accumulated so far tend to demonstrate that infrared spectroscopy could be a very accurate descriptor of the mode of action of anticancer drugs. If confirmed, such a segmentation of potential drugs according to their "mode of action" will be invaluable for the discovery of new therapeutic molecules. We will use here the wording "mode of action" without quotation marks to refer to the mechanism by which the anticancer drugs interfere with the biochemical reactions in the cells.

2. Why a mode of action-based classification of anticancer drugs

As noted by Derenne et al. [67], spectacular advances in our understanding of the molecular and cellular biology of cancer have been seen in the last decades. However, this knowledge has so far not been translated into major improvements in therapy and long-term survival for many cancers [68]. The number of new agents for the treatment of cancer approved by the Food and Drug Administration (FDA) has steadily decreased over the past 10 years [69]. In addition, only 5% of cancer drugs entering clinical trials reach marketing approval and the failure often occurs very late in the clinical development process. The cost of bringing a new anticancer drug to market is thus over US$ 1 billion [68,70]. Successful identification of novel effective anticancer drugs is

Abbreviations: IR, Infrared; FTIR, Fourier Transform Infrared; h, hours; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; SM, sphingomyelin; CL, cardiolipin; CH, cholesterol; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; HPLC, high performance liquid chromatography; TLC, thin layer chromatography; PLS, partial least square.

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presently largely dependent on the use of in vitro assays which are used to obtain a primary selection of active compounds. The US National Cancer Institute (NCI) introduced a screening system based on a panel of 60 cell lines derived from all the major human neoplasms, supposedly representing the heterogeneity of human tumors. Compounds are tested for their ability to inhibit the growth of, or to kill the cells in a 48 or 72 h assay. These tests define, among other parameters, the IC_{50} (growth inhibition), i.e. the negative $\log_{10}$ concentration required to inhibit the growth of a cell line by 50%. [71–73]. The value of the $IC50_{50}$ largely determines whether the molecule will be further investigated. The present situation indicates that this approach is not sufficient. This can be understood as molecules with new interesting modes of action are not likely to be the most cytotoxic/cytostatic ones and are therefore discarded at early stage of the screening.

As already pointed out by Gasper et al. [74,75], obtaining an accurate signature of drug-induced biochemical changes could help identify these molecules which have new, undocumented behaviors. Many new techniques are available for typing the actual chemical content of cells, in particular the –omic approaches. They all limited by reproducibility issues or are complicated, long and costly. IR spectroscopy could become a valuable tool in this domain. It does not require any staining or chemical reagents nor complicated cell handling (i.e. disruption of the membrane, binding of biomarkers, interaction with probes etc.…). A schematic view of the concept discussed in this paper is provided in Fig. 1. This review will investigate the feasibility of using IR spectroscopy to screen and classify drugs according to modes of action.

3. Infrared spectrum provides a global accurate cell signature

As described previously [76] FTIR spectroscopy is based upon the interaction between the IR radiation and the covalent bonds of molecules. IR spectroscopy exploits the fact that molecules have specific frequencies at which they rotate or vibrate corresponding to discrete energy levels (vibrational modes). Within the mid-infrared range (4000–400 cm^{-1} or 2.5–25 μm), all organic functions lead to specific IR absorption bands. Each compound has a characteristic set of absorption bands in its infrared spectrum. It is now considered that the FTIR spectrum provides as much information as DNA microarrays as far as diagnostic purposes are concerned. Importantly, all molecular types contribute to the IR spectrum and this contribution depends on the exact molecular structure. For instance, the head group, length and unsaturation of membrane lipids, all contribute to the IR spectral signature [77,78]. Similarly, lipid/protein ratio, DNA conformation state and many other parameters can be obtained from the spectra [79–84]. Besides, the IR spectra account not only for the chemical nature of cell molecules but also for their conformations and are, in particular, very sensitive to protein secondary structure [11,48,85–110]. All together, the various contributions to the FTIR spectrum form a signature of the biochemical composition of the cell that is unique.

Fig. 2a illustrates the absorbance spectrum of the most abundant molecules present in a cell: proteins, lipids and nucleic acids: RNA and DNA. Nucleic acids have been studied in particular by Wood et al.. They were able to both quantify DNA in simple eukaryotic cells using Fourier transform infrared spectroscopy [82] and to evaluate the B to A DNA conformational change in biological samples [83,84]. The most abundant molecule in the cell is water, which is very intense and masks important spectral regions. Experiments are routinely carried out after dehydration of the cells and the effect of dehydration has been discussed at length, in particular on the structure of proteins. There is a general consensus that the original structure is generally retained [87,96,110–112]. Fig. 2b illustrates the dependence of the protein IR spectrum on the secondary structure. It can be observed that the shape and position of amide I band and amide II bands is strongly dependent on the secondary structure, a feature largely used to determining protein secondary structure as mentioned above. Lipid hydrocarbon chains also have a marked dependence on the presence of unsaturation (Fig. 2c), on the length of the hydrocarbon chain [77] and on the nature of the polar head groups for membrane lipids (Fig. 2d) [78]. Finally, glycosylation also produces very specific spectral signatures (Fig. 2e). These examples suggest that infrared spectroscopy could generate a spectral signature of the cell that is very sensitive to small variations in chemical content and even in the three dimensional structure of biomolecules such as proteins.

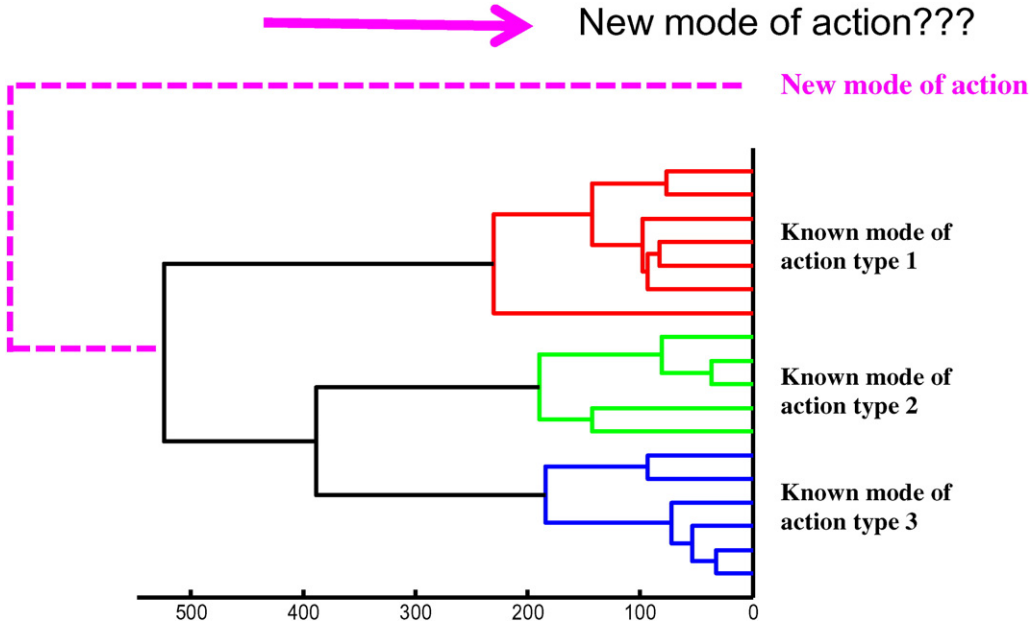

Fig. 1. Schematic figure describing the concept to search for new modes of drug action using FTIR spectroscopy. The dendrogram groups infrared spectra of cells exposed to various anticancer drugs reflecting the similarity of changes induced by the drugs. This similarity in the changes apparent in their FTIR spectra is the signature of one mode of action. Here, three types of modes of actions result in three groups characterized by a specific set of spectral changes. This is for instance illustrated later in this review by molecules targeting topoisomerase II, microtubules and DNA synthesis. The abscissa reports the distance between the spectra, typically the Euclidian distance or Pearson's correlation coefficient is used as the measure of similarity and dissimilarity. In this example the magenta molecule displays a completely different mode of action. This is the type of molecules that should be investigated further, even if the IC_{50} is too low to attract the interest of usual pharmacology studies.