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Evaluation of the characteristics of some plant polyphenols as molecules intercepting mitoxantrone

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ABSTRACT

This paper contains the results of a study on the interactions between mitoxantrone and some bioactive polyphenols. It has been demonstrated that polyphenols can intercept mitoxantrone. Quercetin shows the highest affinity for complexing with mitoxantrone, in contrast to resveratrol, which shows the lowest affinity. The main process underlying the association between cytostatic and polyphenols occurs in the ground state. The values of the constants of the association reactions between the analysed compounds and mitoxantrone are large enough to generate an evident intercepting effect in the three-component system (mitoxantrone-DNA-polyphenol). The affinity of the analysed plant-origin compounds is approximately 1000-fold weaker than the interaction of mitoxantrone with the DNA, which implies that the presence of these compounds in food should not adversely affect oncological therapy but rather could successfully aid oncological treatment by regulating the quantities of the drug in its active form.

1. Introduction

Neoplastic diseases are a grave social problem and one of the major causes of death worldwide (Stewart & Wild, 2014). Methods of treating neoplasms vary depending on their type and typically involve one or many approaches, such as surgery, radiotherapy or chemotherapy. Chemotherapy is one of the most efficient methods used to combat neoplastic cells. Unfortunately, the drugs used for chemotherapy are not selective and destroy healthy cells as well, which can lead to cachexia. The side effects of chemotherapy can be mild or severe and depend on the type of medication administered and the individual response of the patient. The most common unwanted effects of chemotherapy reported by oncological patients are fatigue, nausea, hyperemesis, diarrhoea and hair loss. In addition to selecting an appropriate method for treatment of a neoplastic illness, it is also important to improve the quality of life of oncological patients, which can be achieved by supportive or palliative care and psychotherapy. Because of frequent gastrointestinal tract disorders, an adequately designed diet is an essential component of anti-cancer therapy. Since the 1980s, much attention has been paid to the development and marketing of functional foods. The term 'functional foods', which denotes foodstuffs with special beneficial functions, was coined in Japan (Bultosa, 2016; Kaur & Das, 2011). Broad-scale research has been conducted on functional foods, including examination of the bioactive properties of natural compounds, the effects of these foods on human health such as the pro-health benefits derived from their consumption and the processing and production of food that preserves the biological activity of natural compounds. (Ayseli & Ayseli, 2016; de Boer, Urlings, & Bast, 2016; Marsanasco, Márquez, Wagner, Chiaramoni, & Alonso, 2015; Schröder, 2003; Vieira da Silva, Barreira, & Oliveira, 2016).

The beneficial influence of many natural substances contained in plant products on the human body has been known for centuries. Recent studies have demonstrated that increased consumption of fruit and vegetables decreases the risk of developing neoplasms. Plants comprise many antineoplastic phytocompounds, whose potential bioactivity can reduce the susceptibility to neoplastic diseases. There are many potential mechanisms of action for these compounds (Birt, Hendrich, & Wang, 2001, and inclusive references). The studies performed thus far indicate that one of the mechanisms through which natural compounds act against neoplasms involves their ability to directly intercept particles of mutagens, thereby preventing intercalation (Buchelnikov et al., 2012; Osowski, Pietrzak, Wieczorek, & Wieczorek, 2010; Pietrzak, Wieczorek, Wieczorek, & Darzynkiewicz, 2006; Piosik, Wasilewski, Woziwodzka, Śledź, & Gwizdek-Wiśniewska, 2010). Intercalation is a process by which a flat system of aromatic rings

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of a mutagen is incorporated directly between a pair of bases in the double-stranded helix of nucleic acid; as a result, the DNA helix becomes elongated and locally relaxed, which can lead to mutation (Nafisi, Saboury, Keramat, Neault, & Tajmir-Riahi, 2006; Takagi, 2001). There are many mutagens and antineoplastic drugs among intercalation agents. An example is mitoxantrone, a synthetic antineoplastic analogue of anthracycline antibiotics, which demonstrates high clinical efficacy in the treatment of various neoplasms and multiple sclerosis. Structurally, mitoxantrone is a symmetrical chromophore, comprising a flat anthraquinone ring with two side-chains containing nitrogen. It is actually this flat anthraquinone ring that is responsible for the drug's intercalation between pairs of DNA bases, whereas the side groups interact electrostatically with the negatively charged DNA phosphate skeleton. In physiological pH, mitoxantrone is positively charged (Bhattacharyya, Basu, & Kumar, 2014; Enache, Ionescu, & Volanschi, 2015). Analogous to other anthracyclines, mitoxantrone is characterized by an undesirable, adverse cardio-toxic effect. Presently, the recommended maximum lifetime cumulative dose of mitoxantrone is 140 mg/m^2, with 2.6–13% of patients developing cardiac toxicity at that dose (Dores-Sousa et al., 2015; Wilkes & Barton-Burke, 2016).

In view of the above studies, it is of value to apply natural compounds extracted from plants that possess interceptive activity in order to minimize the toxicity of mitoxantrone and other antineoplastic drugs and to protect healthy cells (for instance, in the gastrointestinal tract), thus reducing the side effects of antineoplastic therapy. In addition, it is essential to maintain the interceptor-intercalator association constants at levels that are low enough not to inhibit the medicinal properties of administered chemotherapeutic preparations.

When binding to DNA intercalators, compounds with interceptive activity form layered sets, called "sandwich-type aggregates" (Evstigneev, 2013). Interactions between the layers result in a change in the distribution of the electron density in the intercalator's molecules. Consequently, changes in the photophysical properties of these molecules are observed, which provides an opportunity to determine the association constants of formed complexes and establish the mechanism of their formation via spectroscopic methods. The selection of appropriate natural compounds that will perform the role of interceptors should rely *inter alia* on their spectroscopic characteristics. It is important that absorption and emission spectra of the intercalator and interceptor lie in different spectral ranges, as this enables selective excitation to be performed and changes occurring within only one type of molecule to be observed. Mitoxantrone is characterized by a long-wave spectrum of absorption and emission and, therefore, natural polyphenolic compounds can serve as appropriate interceptive molecules. Polyphenolics have been detected in many plants, where they function as secondary plant metabolites, usually involved in defence against ultraviolet radiation and pathogenic infestation. Polyphenols are organic compounds that contain at least two hydroxyl groups attached to an aromatic ring. It is precisely due to the presence of the –OH group, which reduces free radicals, that the polyphenols demonstrate antioxidant activity. Polyphenols are a group of compounds that are distinguished by highly varied structures and properties. Plant polyphenols include phenolic acids, flavonoids, stilbenes and lignans (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004; Pérez-Jiménez, Neveu, Vos, & Scalbert, 2010).

Four flavonoid compounds, quercetin, catechin, epigallocatechin gallate and rutin, were used in the present study, as well as resveratrol, a representative of stilbenes produced by some plants. The objective was to determine the mechanism of action, establish the constants for the association of the selected polyphenols with mitoxantrone and determine the impact on the intercalation of mitoxantrone into the DNA in the following three-component system: intercalator – DNA – interceptor.

2. Material and methods

2.1. Chemicals

Mitoxantrone (1,4-dihydroxy-5,8-bis[2-[(2-hydroxyethylamino)ethylamino]-anthracene-9,10-dione), calf thymus DNA, HEPES (*N*-(2-hydroxyethyl)piperazine-*N*'-(2-ethane sulfonic acid), dimethyl sulfoxide (DMSO), quercetin (QCT), epigallocatechine galate (EGCG), rutin (RUT), resveratrol (3,4,5-trihydroxy-*trans*-stilbene, RES) and catechin (CAT) were purchased from Sigma Chemical Co, St. Louis, MO, USA. All compounds were used without further purification. Water used for sample preparation was deionized in an HLP 5UV system produced by HYDROLAB (Gdansk, Poland).

2.2. Sample preparation

The solutions for the experiment were prepared in 20 mM HEPES buffer (pH 7) containing 150 mM NaCl immediately before the experiment. The pH of the buffer solution was adjusted with 1 M NaOH solution using a JENWAY 3030 pH meter calibrated with commercial standard buffers. The mitoxantrone solution was prepared at a low concentration ($\approx 10^{-5}$ M) to depress the formation of dimers and higher aggregates. Due to their low solubility in water, quercetin, rutin and catechin were dissolved in a small volume of DMSO. The resultant stock solution was then mixed with HEPES buffer until solutions with desired concentrations were reached. The total concentration of DMSO in a cuvette was 3% and remained constant. The stability and DNA concentration in probes were checked spectrophotometrically before each series of measurements. The DNA concentration in moles of nucleotides per litre was constant and determined using the absorption coefficient $\varepsilon_{(260nm)} = 6600\ M^{-1}cm^{-1}$ (Zhen et al., 2000).

2.3. Spectroscopic measurements

Absorption spectra were recorded using a Cary 5000 UV–Vis-NIR spectrophotometer (Varian Inc., Australia). All measurements were performed in 22° C. Mitoxantrone absorption spectra were measured as a function of the polyphenol concentration at room temperature. The measurements were carried out in a 3 cm cuvette and a wavelength range of 230–800 nm. The self-association constants K_{MT} and the absorption spectra of mitoxantrone in the monomeric form and complexes were estimated numerically.

Fluorescence measurements were performed using a Cary Eclipse spectrophotometer (Varian Inc., Australia), whose cell-housing block was thermostated. The concentrations and temperature were the same as for the absorption measurements. The excitation wavelength was 610 nm. To avoid reabsorption, 0.4 cm cuvettes were used, and the optical density of a sample at the excitation wavelength did not exceed 0.1.

Fluorescence decays were measured with time correlated single photon counting equipment (FluoroTime 200, PicoQuant GmbH, Berlin, Germany) equipped with an R3809U-50 microchannel plate photomultiplier (MCP-PMT, Hamamatsu) and a PicoHarp300 TCSPC module. Excitation was achieved with a pulsed 635 nm laser diode (LDH-P-635) driven by a PDL800-D driver. The instrument response function (IRF) was recorded with Ludox showing full width at half maximum (FWHM) around 90 ps.