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The effect of quercetin on the electrical properties of model lipid membranes and human glioblastoma cells

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ABSTRACT

Quercetin is a naturally-occurring flavonoid claimed to exert many beneficial health effects. In this report, the influence of quercetin on the surface charge of phosphatidylcholine liposomes and human glioblastoma LN-229 and LN-18 cells was studied using microelectrophoretic mobility measurements. The effect of quercetin on the electrical resistance and capacitance of bilayer lipid membranes was analyzed via electrochemical impedance spectroscopy. The results showed that after flavonoid treatment, the cell lines demonstrated changes in surface charge only in alkaline pH solutions, whereas there were no significant alterations in quercetin-treated vs. control cells in acidic pH solutions. The same tendency was found for liposomal membranes proving that quercetin insertion into membranes is strongly pH-dependent. Capacitance and resistance measurements conducted in acidic electrolyte solutions demonstrated an increase in both electrical parameters, indicating an increased amount of quercetin inserted into the bilayers. Moreover, the cytotoxic effect of quercetin confirms that the flavonoid enters the cells and perturbs the proliferation of LN-229 and LN-18 glioblastoma cell lines. As such, our results indicate that the specific localization of quercetin, membrane-bound or cell-entering, might be crucial for its pharmacological activity. However, further studies are necessary prior to applying these physicochemical measurements as standard methods of evaluating drug activity.

1. Introduction

The living cell is a dynamic system concerned with the processes of energy capture, transfer and conversion. Each activity is complex, consisting of myriads of individual integrated biochemical reactions that collectively constitute the key processes determining cellular functions. Each cell type is unique in terms of its structure and function, and its own characteristics are established by the architecture of its components [19].

There have been numerous attempts to use electrochemical techniques in cell biology, but they have a few common problems to overcome. The first one, is that electrochemistry often requires a very clean protein-free media, while cells require proteins in culture medium to maintain their biological activities. The second drawback is connected with low reproducibility of experimental results caused by non-homogenous biological materials used in studies and complexity of the experimental procedures. Finally, the determination of what is actually measured in very complex biological systems may sometimes cause additional problems [15]. One of the most important electrochemical techniques in characterization of functions and physical properties of biological membranes is electrochemical impedance spectroscopy (e.g. [2, 26, 38]).

It has been evident, that the lipid bilayer is the major structural component of biological membranes. Therefore, knowledge concerning properties and formation of these structures in vitro presents considerable significance, on both experimental and theoretical levels. It is readily apparent that a detailed physical and chemical description of biological membranes may be best approached by studies of simplified well-defined models [55]. Many examples of commonly used models imitating different biological membranes can be found in the literature. The most frequently employed are planar lipid bilayers, called black lipid membranes (BLMs), formed across a hole in a partition between two aqueous solutions, and bilayers made in the form of spherical vesicles, called liposomes. The geometry of BLMs enable chemical as well as electrical access to both sides of the bilayer, and allows to easily measure the physical attributes of these bimolecular films. Two electrical properties of BLMs are particularly important: the membrane capacitance C_m and the membrane resistance R_m. The capacitance is considered to be the best tool for recording the stability and integrity of

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bilayers. For comparison between different studies, the measured capacitance must be standardized to the size of the bilayer surface and is usually given as capacitance per unit area [22]. The resistance is usually calculated in accordance with Ohm's law as the ratio of voltage applied to the bilayer and a current flowing through it [22]. The resistance may vary by at least one order of magnitude for different membranes. However, R_m of a single membrane is usually constant, therefore any changes in resistance value caused by the presence of additional substances can be determined with a relatively high degree of accuracy [55]. Liposomes are composed of an inner aqueous compartment surrounded by lipid bilayers, the membranes of which are formed by natural or synthetic lipids [28]. These models are primarily categorized into tree types: multilamellar vesicles, small unilamellar vesicles and large unilamellar vesicles, with sizes ranging from nanometers to micrometers in diameter [14]. Liposomes can be formed spontaneously when phospholipids or other amphiphilic molecules with specific properties are hydrated [40]. The lipids in liposomes can undergo various degrees of charging in aqueous solutions at various pH ranges [27]. The zeta potential (electrophoretic mobility) and surface charge are important parameters which describe the electrical properties of liposomes surface. Thus, the electrostatic interaction was demonstrated to play an important role in drug-liposome binding [24]. A relatively fast determination of these parameters can be done using laser Doppler velocimetry. This technique allows the application of an electric field to the measuring vessel, resulting in movement of liposomes within the vessel. The mobility of the liposomes is proportional to their charge, and their movement evokes a Doppler shift in frequency of the detected laser light. Thus, zeta potential is subsequently calculated from the frequency of fluctuations of the detected light [39, 58].

Contemporary medical and pharmacological studies focus on employing interdisciplinary approaches for successful therapeutic outcomes. Thus, the combination of physicochemical and cell-based methods in describing potential drug properties should become a widely-applied trend in modern biomedicine. In this regard, the aim of this work was to explore the interactions between bilayer lipid membranes, liposomes and human glioblastoma LN-229 and LN-18 cells with quercetin (QCT) – a naturally produced plant polyphenol [52]. The chemical structures of quercetin and phosphatidylcholine (PC) – a lipid component of model membranes are shown in Fig. 1. In order to cover all aspects of membrane-QCT interactions, three different experimental approaches were applied. The BLMs and the liposomes were used as models of (artificial) cell membranes, while glioblastoma cells served as a predictor of QCT functioning in living cells. In particular, the ability of quercetin to alter the surface charge of liposomes and cultured cells as well as the electrical resistance and capacitance of BLMs was addressed by employing microelectrophoretic mobility measurements and electrochemical impedance spectroscopy.

There is now much interest in using plant polyphenols in cancer chemoprevention. They are considered to be safe and highly effective agents in inhibiting of carcinogenesis. The compounds derived from the plants are of special interest among oncologists and pharmacists [50]. One of such compounds is quercetin. Quercetin (3,3′,4′,5,7-pentahydroxyflavone) belongs to the group of bioflavonoids and is a typical polyphenolic compound widely distributed in vegetables and fruits [52]. The available data indicate, that quercetin possesses many beneficial effects on human health such as protection against osteoporosis as well as cardiovascular and pulmonary diseases, and also against some aging symptoms [50]. Many of these effects are supposed to be related to its anti-apoptotic, anti-inflammatory and antioxidant properties [8, 31]. Furthermore, QCT can inhibit growth and proliferation of a variety of cancer cell lines including ovarian, colon, lung, and breast cancer cells [13]. It can also lower the multidrug-resistance and enhance the antitumor effects of other chemotherapeutic drugs [48, 56]. While a considerable amount of data concerning QCT effects in various types of cancers exist, there is limited amount of studies reporting quercetin efficiency in brain tumors. Unlike other cancers, brain tumors are particularly inaccessible to chemotherapeutics due to the blood-brain barrier, and a number of other factors limiting the efficacy of available treatments [25]. Therefore, glioblastoma still poses a therapeutic challenge with poor overall prognosis. To address the need for new therapeutic strategies, application of natural compounds such as QCT is currently being explored in the management of brain malignancies. To date, several reports illustrating the utility of QCT for brain tumor treatment have been published. It has already been demonstrated that QCT sensitizes human glioblastoma U251, U87 and DBTRG-05 cells to temozolomide – an alkylating agent commonly used in anti-glioblastoma therapy [43, 47]. Moreover, it has been evidenced that QCT reduced proliferation and migration of human glioblastoma U251 cells [29], and initiated apoptotic death in glioblastoma T98G cells [20].

Although certain amount of information concerning cellular and molecular effects of QCT in brain malignancies has already been available, the mechanisms of interaction between quercetin and biological membranes has not been fully understood yet, and the data presented so far remains controversial. Still, the issue of membrane integrity and permeability seems to be of great importance in context of drug efflux and effectiveness of pharmacotherapy. Thus, the aim of these studies was to perform the first systematic research investigating the effect of quercetin on electrical properties of both, model biological membranes and the membranes of living cells (together with the attempt of correlating these changes with alterations in viability of glioblastoma cells).

2. Materials and experimental details

2.1. Reagents

1,2-Diacyl-*sn*-glycero-3-phosphocholine (99%), quercetin (≥95%) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide were provided by Sigma (St. Louis, MO, USA). The phosphatidylcholine had the following fatty acid composition: 16:0 ~33%, 18:0 ~4%, 18:1 ~30%, 18:2 ~14%, and 20:4 ~4%. The molecular weights of the phosphatidylcholine and quercetin were 752.08 and 302.24 g/mol, respectively. The Dulbecco's modified Eagle's medium (DMEM), containing glucose at 4.50 mg/cm^3 with GlutaMaxTM, trypsin-EDTA, penicillin, streptomycin and fetal bovine serum Gold (FBS Gold) were provided by Gibco (San Diego, CA, USA). All other chemicals were of the best quality commercially available. The solutions and cleaning procedures were performed using triple-distilled water, the second distillation was

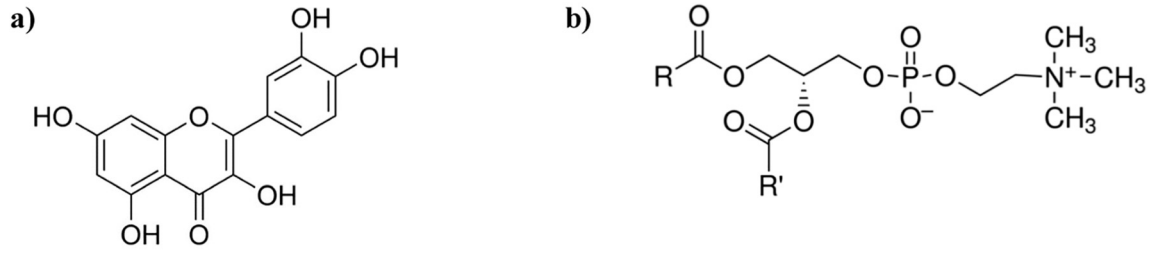

Fig. 1. The molecular structure of quercetin (a) and phosphatidylcholine (b).