Chemico-Biological Interactions 282 (2018) 29–35

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Effects of rutin on the physicochemical properties of skin fibroblasts membrane disruption following UV radiation

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ARTICLE INFO

Keywords:
Rutin
UV radiation
Surface charge density
Sialic acid
Lipid peroxidation
Fibroblasts

ABSTRACT

Human skin provides the body's first line of defense against physical and environmental assaults. This study sought to determine how rutin affects the membrane electrical properties, sialic acid content, and lipid peroxidation levels of fibroblast membranes after disruption by ultraviolet (UV) radiation. Changes in cell function may affect the basal electrical surface properties of cell membranes, and changes can be detected by electrokinetic measurements. The charge density of the fibroblast membrane surface was measured as a function of pH. A four-component equilibrium model was used to describe the interaction between ions in solution and ions on the membrane surface. Agreement was found between experimental and theoretical charge variation curves of fibroblast cells between pH 2.5 and 8. Sialic acid content was determined by Svennerholm's resorcinol method, and lipid peroxidation was estimated by measuring the malondialdehyde level. Compared to untreated cells, ultraviolet A (UVA)- or ultraviolet B (UVB)-treated skin cell membranes exhibited higher concentrations of acidic functional groups and higher average association constants with hydroxyl ions, but lower average association constants with hydrogen ions. Moreover, our results showed that UVA and UVB radiation is associated with increased levels of sialic acid and lipid peroxidation products in fibroblasts. Rutin protected cells from some deleterious UV-associated membrane changes, including changes in electrical properties, oxidative state, and biological functions.

1. Introduction

The skin plays a critical role in protecting humans during daily exposure to external physical and chemical factors, including ultraviolet (UV) radiation from sunlight. Human skin is regularly exposed to ultraviolet B (UVB; 280–320 nm) and ultraviolet A (UVA; 320–380 nm) radiation, whereas ultraviolet C (UVC; 100–280 nm) radiation is blocked by the ozone layer. Although these three types of radiation generate different biological effects, they both enhance the levels of reactive oxygen species within cells and tissues [1]. Moreover, UVA leads to endogenous photosensitizers activation, in addition to ROS generation [2]. Such situation may lead to protein amino acid side-chain reactions (including oxidation and crosslinks formation), DNA oxidative damages and lipid peroxidation and then to reactive endogenous electrophiles generation.

Cell membranes, comprising phospholipids, sphingolipids, glycoproteins, glycolipids, and cholesterol, are greatly affected by exposure to UV radiation. Carbohydrate portions of membrane glycoproteins and glycolipids, which predominantly contain sialic acid, project from the outer cell membrane surface and form the cell coat. Sialic acid plays important roles in cell-cell recognition, invasiveness, adhesion, and immunogenicity [3,4].

Phospholipids play important roles as immunomodulators and signaling molecules [5]. These molecules promote the appropriate hydrophobic environment for major cellular events at the cell membrane interface, such as ligand-receptor interactions, endocytosis, and antigen presentation [6].

Changes in cell membrane components can influence the electrical properties of the membrane and the equilibrium between the cell membrane and its environment. Any perturbation in cell function, including exposure to UV radiation, can lead to variations in electrical properties of the phospholipid bilayer. Thus, measurements of membrane electrical charge can provide important information on the equilibrium within the cell membrane and between the membrane and

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https://doi.org/10.1016/j.cbi.2018.01.012
Received 25 August 2017; Received in revised form 28 December 2017; Accepted 11 January 2018
Available online 12 January 2018
0009-2797/

its environment, under physiological and nonphysiological conditions [7,8]. Changes caused by UV radiation can be monitored by determining the electrical charge of the membrane as a function of environmental pH, the concentrations of acidic and basic functional groups (C_{TA} and C_{TB}, respectively), and their average association constants with hydrogen or hydroxide ions (K_{AH} and K_{BOH}, respectively).

Researchers continue to study the efficacies and mechanisms of action of biologically relevant antioxidants, such as polyphenolic compounds, to identify compounds that can overcome the deleterious consequences of UV radiation. Recently, the antioxidative and photoprotective properties of polyphenolic compounds have been receiving increasing attention for their potential use as nutrients or in topical applications. One large group of polyphenols is the flavonoids, which include the quercetin glycoside rutin [9].

The purpose of this work was to determine the influence of rutin on the electrical properties, lipid peroxidation, and sialic acid content of fibroblast membranes following membrane disruption by UV radiation. The quantitative description of cell membrane properties can aid in interpreting and understanding the processes that occur on biological membranes during UV radiation.

2. Theory

The model, which has been presented in full detail in a previous study [10,11], assumes that dependence of surface charge density of the cell membrane on the pH of the electrolyte solution can be described via four equilibria.

Assumptions of the model are described by Eqs. (1)–(7). The electrolyte solution ions (H^+, OH^-, Na^+, and Cl^-) are adsorbed at the cell membranes of fibroblasts. There are two equilibria for negative groups, with sodium and hydrogen ions, and two equilibria for positive groups, with hydroxide and chloride ions. The adsorption equilibria (equations (1)–(4)), the surface charge density (equation (5)) and the functional group concentration balances (equations (6) and (7)) are presented as follows:

$$A^- + H^+ \Leftrightarrow AH \quad (1)$$

$$A^- + Na^+ \Leftrightarrow ANa \quad (2)$$

$$B^+ + OH^- \Leftrightarrow BOH \quad (3)$$

$$B^+ + Cl^- \Leftrightarrow BCl \quad (4)$$

$$\delta=(a_{B^+}+ a_{A^-})\cdot F \quad (5)$$

$$C_{TA} = a_{A^-} + a_{AH} + a_{ANa} \quad (6)$$

$$C_{TB} = a_{B^+} + a_{BOH} + a_{BCl} \quad (7)$$

where a_{A^-}, a_{AH}, a_{ANa}, a_{B^+}, a_{BOH}, and a_{BCl} are the surface concentrations of the corresponding groups on the membrane surface; and a_{H^+}, a_{Na^+}, a_{OH^-}, and a_{Cl^-} are the corresponding concentrations in solution; C_{TA} is the total surface concentration of the acidic groups and C_{TB} is the total surface concentration of the basic groups; F= 96487 $\left[\frac{C}{mol}\right]$ is the Faraday constant; δ – the surface charge density.

Final equations describing surface charge density of the membrane [10]:

$$\frac{\delta}{F} = \frac{C_{TB}\cdot a_{H^+}}{a_{H^+}(1 + K_{BCl}\cdot a_{Cl^-}) + K_{BOH}\cdot K_w} - \frac{C_{TA}}{K_{AH}\cdot a_{H^+} + K_{ANa}\cdot a_{Na^+} + 1} \quad (8)$$

where K_{AH}, K_{ANa}, K_{BOH}, and K_{BCl}-are the association constants.

It is difficult to solve equation (8) and determine the C_{TA}, C_{TB}, K_{AH}, and K_{BOH} constants. Liner equation obtained by simplification of Eq. (8) valid for high (Eq. (9)) and low (Eq. (10)) concentration of hydrogen ions:

$$\frac{\delta}{F}a_{H^+} = \frac{C_{TB}}{1 + K_{BCl}\cdot a_{Cl^-}}\cdot a_{H^+} - \left(\frac{K_{BOH}\cdot K_w\cdot C_{TB}}{(1+K_{BCl}\cdot a_{Cl^-})^2} + \frac{C_{TA}}{K_{AH}}\right) \quad (9)$$

$$\frac{\delta}{F}a^1_{H^+} = \frac{-C_{TA}\cdot a^1_{H^+}}{1 + K_{ANa}\cdot a_{Na^+}} + \left(\frac{C_{TB}}{K_{BOH}\cdot K_w} + \frac{K_{AH}\cdot C_{TA}}{(1+K_{ANa}\cdot a_{Na^+})^2}\right) \quad (10)$$

The coefficients estimated from the linear regression can be used to determine C_{TA}, C_{TB}, K_{AH}, and K_{BOH}. The points included in the regression must be carefully selected, both in high and low pH ranges. Defining the value of these parameters permits the calculation of the theoretical cell membrane surface charge from equation (8) for comparison to experimental data.

3. Materials and methods

3.1. Cell culture and treatment

Human fibroblasts (CCD 1112Sk) obtained from the American Type Culture Collection were cultured in a humidified atmosphere of 5% CO_2 at 37 °C in Dulbecco's Modified Eagle Medium (DMEM) contained fetal bovine serum (10%) and supplemented with 50 U/ml penicillin and 50 μg/ml streptomycin. When the cells (passage 8) reached 70% confluence, they were exposed to UV radiation. The cells were irradiated from the 6 lamps (Bio-Link Crosslinker BLX 312/365; Vilber Lourmat, Germany) assembly 6 W each, which corresponds to 4.2 mW/cm^2 and 4.08 mW/cm^2, respectively for UVA (365 nm) and UVB (312 nm). Total radiation doses were 20 J/cm^2 and 200 mJ/cm^2 for UVA and UVB respectively. UV radiation doses were chosen to correspond to 70% cell viability. The concentration of rutin (25 μM) was selected based on 95% cell viability measured by the MTT assay [12] (data not shown). This concentration of rutin was previously shown to have significant effects on intracellular signaling and cellular redox status [13]. To determine the effects of rutin on UVA/UVB-irradiated fibroblasts, several experimental groups were examined.

Control group cells were cultured in medium containing 0.2% DMSO. *Rutin group* cells were incubated in medium containing 25 μM rutin (in 0.2% DMSO solution). The *UVA group* or *UVB group* cells were irradiated by UVA or UVB, respectively, and then cultured in medium containing 0.2% DMSO. The *UVA + rutin group* or *UVB + rutin group* cells were irradiated with UVA or UVB, respectively, and then cultured in medium containing 25 μM rutin (in 0.2% DMSO solution). The *rutin + UVA + rutin group* or *rutin + UVB + rutin group* cells were pretreated with rutin for 12 h, irradiated with UVA or UVB, respectively, and then cultured in medium containing 25 μM rutin (in 0.2% DMSO solution). For all groups, after incubation for 2 h, cells were washed with PBS, collected by scraping into cold PBS, centrifuged, resuspended in PBS, and sonicated.

3.2. Electrochemical method

An electrochemical method was used to determine the charge density of the cell membrane surface. Cells suspended in 0.9% NaCl were placed in a measuring vessel, and electrophoretic mobility was measured as a function of pH with a Zetasizer Nano ZS apparatus (Malvern Instruments, Malvern, UK). Surface charge density was calculated by using the equation σ = ηu/d, where u is electrophoretic mobility and η is solution viscosity. Thickness of the diffuse layer, d [14], was determined from formula [15] d= $\sqrt{\frac{\varepsilon\cdot\varepsilon_0\cdot R\cdot T}{2\cdot F^2\cdot I}}$, where R is the gas constant, T is temperature, F is the Faraday number, I is the ionic strength of 0.9% NaCl, and $\varepsilon\cdot\varepsilon_o$ is the permeability of the electric medium.

3.3. Sialic acid content

The sialic acid content of membranes was determined by the modified Svennerholm's resorcinol method, which quantifies the total content of sialic acid, whether free or glycoside-bonded [16]. Color intensity was measured at 630 nm. The sialic acid concentration was read