Full Length Research Paper

Quercetin and epigallocatechin gallate in vitro induced changes in the membrane anisotropy of peripheral blood mononuclear cells from patients with inflammatory diseases

Denisa Margina1*, Mihaela Ilie2, Carolina Negrei2, Daniela Gradinaru1, Andra Balanescu3 and Niculina Mitrea1

1Department of Biochemistry, Faculty of Pharmacy, Carol Davila University of Medicine and Pharmacy, 6, Traian Vuia Street, 020956, Bucharest, Romania.
2Department of Toxicology, Faculty of Pharmacy, Carol Davila University of Medicine and Pharmacy, 6, Traian Vuia Street, 020956, Bucharest, Romania.
3Faculty of Medicine, Research Center on Rheumatic Diseases, Carol Davila University of Medicine and Pharmacy, 37-39 Bl. Ion Mihalache, 011195, Bucharest, Romania.

Accepted 18 October, 2010

The aim of the study was to assess the in vitro effect of quercetin and epigallocatechin gallate on the membrane fluidity of mononuclear blood cells isolated from patients with an inflammatory profile (chronic hyperglycemia and rheumatoid arthritis). The measurements were performed via the evaluation of the fluorescence anisotropy for cells labeled with TMA-DPH. Chronic hyperglycemia does not impair the membrane anisotropy, as rheumatoid arthritis does. Quercetin had no significant effect on rheumatoid arthritis cells. Epigallocatechin gallate had a stronger effect, improving the membrane fluidity characteristics for both hyperglycemia and rheumatoid arthritis patients.

Key words: Membrane fluidity, diabetes, quercetin, epigallocatechin gallate.

INTRODUCTION

The flavonoid compounds in vegetable based diets bring a significant contribution to the role of fruits and vegetables as health-promoting foods (Boots et al., 2008). Numerous studies support the antioxidant potential of these phenolic phytochemicals (as free radical scavengers and as inhibitors of enzymes generating reactive oxygen species) and other disease-preventative characteristics, such as antimicrobial properties, anti-inflammatory, anti-cancer activity, cardiovascular-related effects and the capacity to modulate blood glucose (Cheplick et al., 2010).

Quercetin and epigallocatechin gallate are flavonoids largely present green tea, fruits and vegetables that exhibit all the above mentioned properties of vegetal polyphenolic compounds (Erlund, 2004; Xiao et al., 2010; Khai, 2010; Khai, 2010b). The effects of flavonoids have been explained by their binding to, or interference with enzymes, receptors, transporters and signal transduction systems, events that frequently occur in the lipid membrane environments (Middleton, 2000). Therefore, the bioactivity of flavonoids may be attributed to their capacity to interfere with the membrane physical-chemical properties (e.g. anisotropy/fluidity). The behavior of the cell membranes is affected by the physio-pathological settings, oxidative stress and inflammation being factors that deeply impair the membrane anisotropy.

*Corresponding author. E-mail: denisa.margina@gmail.com. Tel: 0040744339630.

Abbreviations: TMA-DPH, 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene p-toluensulfonate; Q, quercetin; EGG, epigallocatechin gallate; PBMC, peripheral blood mononuclear cells; RA, rheumatoid arthritis; HG, hyperglycemia.
Since the great bioactivity of flavonoids is closely linked to their ability to interact with membranes, it is important to characterize their effect on the membrane properties (Oteiza et al. 2005). However, very few systematic studies concerning the effect of quercetin (Q) and epigallocatechin gallate (EGG) on the peripheral blood mononuclear cells (PBMC) membrane properties are found in the literature. Redox stress and inflammation - the main underlying mechanisms of both chronic hyperglycemia and rheumatoid arthritis – strongly impair the properties of the blood cells (Ferrante et al., 2010; Pazdro et al., 2010). In this context, the aim of our study was to assess the effect of Q and EGG on the membrane anisotropy of PBMCs isolated from patients with a well documented pro-inflammatory profile, namely patients with chronic hyperglycemia (HG) and rheumatoid arthritis (RA).

**MATERIALS AND METHODS**

**Reagents**

HPLC grade quercetin (Q) was purchased from MERCK and epigallocatechin gallate (EGG) was purchased from SIGMA. Both have been used for the in vitro stimulation of the cells. 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene p-tolensulfonate (TMA-DPH), used as a membrane anisotropy fluorescence probe, was purchased from Molecular Probes (INVITROGEN INC.).

**Devices**

The membrane fluidity was evaluated via the changes in the membrane anisotropy using a LS50 B spectrofluorimeter (PERKIN ELMER), equipped with thermostated cell holder, magnetic stirring and fluorescence polarization accessory.

**Subjects**

We selected 89 human subjects for the study that were divided into three groups:

1. 29 subjects with a normal metabolic profile (fasting glucose < 110 mg/dL, total cholesterol < 220 mg/dL, triglycerides < 150 mg/dL, normal blood pressure and body mass index), that constituted the control group.
2. 32 patients with chronic hyperglycemia (> 125 mg/dL) that constituted the HG group.
3. 28 patients with rheumatoid arthritis (diagnosed according to the 1987 revised criteria of the American College of Rheumatology, DAS28 score > 5.1), that constituted the RA group.

The informed consent of the subjects was obtained. Patients with severe renal, hepatic or hematological disease, overt cardiovascular disease or malignancy were excluded from the study. None of the subjects had taken known antioxidants-containing supplements (vitamin C, vitamin E, probucol, etc.) 2 weeks prior to the study.

**Biological samples**

Fasting peripheral venous blood samples (on Na2EDTA as anticoagulant) were drawn from the subjects and used for the isolation of mononuclear cells (PBMC) using the gradient density method, with Hypaque 1077 (SIGMA). The cells were standardized at 10^6 cells/mL in RPMI 1640 medium (MERCK) (Ilie et al., 2009). Plasma samples were used for the evaluation of total cholesterol, fasting plasma glucose and triglycerides, using BIORAD enzymatic commercial kits. The general inflammatory marker CRP was assayed with an ELISA kit from DRG INTERNATIONAL.

**Fluorescence anisotropy measurements**

The fluorescence anisotropy of PBMC was evaluated prior and after 20 min of incubation in darkness, at room temperature, with 1 µM Q and 10 µM EGG, respectively. The cell membrane anisotropy measurements were performed using the anisotropy value of TMA-DPH in a steady state fluorescence polarization experiment, after the probe permeates the cell membrane (Khury et al., 1983). For the purpose, the normalized cell suspension labeled with 2.5 µM TMA-DPH (2 min incubation at darkness, at 37°C, under continuous magnetic stirring) was excited with polarized light at 340 nm and the emission intensities were detected at 425 nm, through a polarizer system. The measurements were carried out for unstained cells (as background) and TMA-DPH labeled cells, in the four possible relative positions of the polarizers in the excitation and emission beam; the calculation of the fluorescence anisotropy (r) was performed according to Equation (1):

\[
r = \frac{I_{vv} - GI_{vh}}{I_{vv} + 2GI_{vh}}
\]

where, \(I_{vv}, I_{hh}, I_{vh} \) and \(I_{hv}\) represents the emission intensity, corrected for the background, when the polarisors in the excitation and emission beams are oriented vertical-vertical, vertical-horizontal, horizontal-vertical and horizontal-horizontal, respectively, and \(G = \frac{I_{hh}}{I_{vv}}\) is a correction factor (Lakowicz, 2004). Only the r values in the interval 0 to 0.4 were taken into account, as these are the limiting anisotropy values of the probe itself (Khury et al., 1983).

**Statistical analysis**

Results are expressed as means ± standard deviation (SD). Statistical analyses were performed using the Student t test. Differences were considered significant for \(p < 0.05\).

**RESULTS AND DISCUSSIONS**

Our results showed that hyperglycemia (HG), as well as rheumatoid arthritis (RA), is associated with inflammatory processes. The CRP level was significantly higher in both HG (2.25 ± 0.91 mg/L, \(p < 0.05\)) and RA (4.30 ± 0.40 mg/L, \(p < 0.001\)), compared to controls (1.64 ± 1.12 mg/L). RA and HG have in common in their etiology the inflammatory and redox stress phenomena, which induce and accelerate each other. Exposure to redox stress is documented to induce, both in vitro and in vivo, an increase of membrane peroxidation products levels as...
well as a decrease in membrane fluidity (Reyes et al., 2009; Kamboj et al., 2009).

Epidemiologic studies have reported a reduced risk of cardiovascular disease in subjects with a high flavonoid intake, without exploring all the aspects of the molecular mechanisms. Much of the research in the field was focused on epigallocatechin-3-O-gallate and quercetin, which were found to have beneficial effects against cancer and cardiovascular disease (Jagtap et al., 2009). The plasma membrane of cells is not a homogeneous assembly of molecules, but is organized in many distinct microdomains with different lipid and protein composition, associated with specific functional properties (Monvel et al., 2006). The fluid properties of biological membranes are essential for numerous cell functions including cell growth, solute transport, signal transduction, and membrane-associated enzymatic activities. Even slight changes in the membrane fluidity may cause aberrant functioning and pathological processes (Marczak et al., 2009).

According to literature, the fluorescence anisotropy values are negatively correlated to cell membrane fluidity, thus a high degree of fluorescence anisotropy represents a high structural order or low cell membrane fluidity. Therefore, a decrease in the fluorescence anisotropy is indicative of an increase in the overall membrane “fluidity” (Gramza et al., 2005; Companyo et al., 2007; Shrivastava et al., 2007). TMA-DPH is generally used to monitor fluidity near the surface of the cell membrane. The polar region of this probe is anchored at the lipid–water interface, while the hydrocarbon moiety enters the lipid part of the membrane. The length of the hydrophobic part of the TMA-DPH molecule is approximately equivalent to that of a 10-carbon aliphatic chain and thus can provide information on this region including the surface and glycerol side chain field of the plasma membrane (Marczak et al., 1993). Results shown in Table 1 indicate that the natural occurring products tested in the conditions mentioned above, had not a dramatic effect upon the PBMC membrane fluidity. However, a few facts can be stressed.

A comparison of the results obtained for the anisotropy (r) values showed that unstimulated cells from the HG patients were similar to those of the control group, but for RA patients, the r value was significantly higher than the control group (that is, the unstimulated PBMCs' membrane fluidity of the RA was lower). This was associated with a significant increase of the CRP level, illustrating a profound inflammatory process for the RA patients. We suggest that inflammation induces a decrease of the PBMC membrane fluidity. Although apparently slight, the observed change of the PBMC membrane anisotropy in the RA patients compared to controls (0.31 compared to 0.28) is noteworthy since a lot of experimental data has shown that even small modifications of fluidity can affect membrane functions through the control exerted by phospholipids matrix on membrane protein activities (Beccerica et al., 1988). For the control group, the fluorescence anisotropy of the PBMC was similarly reduced by stimuli. Results show that Q and EGG resulted in an improvement of the membrane fluidity in normally metabolic patients.

For the HG samples, the decrease of the fluorescence anisotropy was double under the effect of EGG compared to Q. Thus, in cells isolated from patients with cardiovascular risk due to endothelial inflammation and redox stress, Q determined a weaker effect on the membrane fluidity of PBMCs compared to that exerted by EGG.

For the cells isolated from the RA patients, Q had virtually no effect on the fluorescence anisotropy, but EGG lead to a significant decrease of the fluorescence anisotropy.

The impairment of the membrane anisotropy, reflected by the results of the present study, associated with pronounced inflammatory phenomena which might lead to an increase of the endothelium-leukocyte interaction probability, explaining the risk of vascular complication especially since cardiovascular disease largely accounts for the increased morbidity and mortality observed in rheumatoid arthritis (Ferrante et al., 2010). According to literature data, RA as well as HG are associated with oxidative stress and, for these pathologies there is evidence of increased serum levels of free radical oxidation products and of decreased levels of free radical scavengers and antioxidant enzyme systems, which normally protect the biological membranes from peroxidation. The redox stress associated with the inflammation could induce the observed changes in lymphocyte membrane fluidity, and these could be either a passive consequence of the disease conditions or be directly involved in their pathogenetic mechanisms (Beccerica et al., 1988; Reyes-Gonzales et al., 2009). In the future, additional experiments can be performed to

### Table 1. Anisotropy values obtained for the PBMCs isolated from HG and RA patients.

<table>
<thead>
<tr>
<th></th>
<th>Unstimulated PBMCs</th>
<th>PBMC stimulated with 1 µM Q</th>
<th>PBMC stimulated with 10 µM EGG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute value</td>
<td>% *</td>
<td>Absolute value</td>
</tr>
<tr>
<td>Control (n=29)</td>
<td>0.28±0.03</td>
<td></td>
<td>0.26±0.04</td>
</tr>
<tr>
<td>HG group (n=32)</td>
<td>0.28±0.08</td>
<td>0.26±0.04</td>
<td>7.1</td>
</tr>
<tr>
<td>RA group (n=28)</td>
<td>0.31±0.08</td>
<td>0.31±0.10</td>
<td>0</td>
</tr>
</tbody>
</table>

* % relative decrease as compared to unstimulated samples.
strength the results outlined in the present article; this will be done by correlation studies of the PBMC membrane fluidity and specific inflammatory markers (ICAM, VCAM, selectins), in order to determine if the impairment of the cell membrane behavior might activate inflammatory phenomena at the endothelial level.

Conclusions

The in vitro study of natural occurring flavonoids (quercetin, epigallocatechin gallate) effect on the membrane fluidity of peripheral blood mononuclear cells from patients with an inflammatory profile (rheumatoid arthritis, chronic hyperglycemia) was performed via the evaluation of changes in the bound-to-membrane fluorescence anisotropy of TMA-DPH. Although, further studies are necessary to elucidate the aspects of membrane changes in association with pathology, we suggest that the lymphocyte membrane can be used to investigate molecular events of RA and HG.

In this study, it was demonstrated that PBMC membrane fluidity was strongly affected by intense inflammatory phenomena associated with rheumatoid arthritis, and less in chronic hyperglycemia as compared to normally metabolic subjects.

Quercetin and epigallocathecin gallate both improved the membrane fluidity at the surface of PBMC isolated from normally metabolic subjects. The effect of Q was reduced on cells isolated from hyperglycemic patients and acute inflammation in rheumatoid arthritis patients lead to the inhibition of Q effect on the membrane fluidity. EGG, on the other hand, improved the membrane fluidity in all types of tested cells, regardless of the metabolic conditions that they were exposed to, showing a stronger effect compared to quercetin. The results suggest that food/supplements with EGG have a better chance of reversing the pathological changes associated with inflammation and redox stress; so EGG more than Q might be used as a strategy aimed at sustained control of membrane anisotropy in patients subjected to systemic inflammatory phenomena, in order to reduce the risk of cardiovascular complications. The two compounds could be used for the prevention of the cell membrane properties impairment in normally metabolic subjects. Further studies are required in order to establish the exact mechanism through which polyphenolic compounds like Q and EGG act on the membrane fluidity. These studies should use multiple fluorescent probes that will show how Q and EGG influence the anisotropy in all the membrane, not only in its exterior segment.

ACKNOWLEDGEMENT

The work was performed under the CNCSIS grant PD 132/30.07.2010 (PD 29/2010).

REFERENCES


