FLAVONOIDS EFFECT ON THE LIPID ORDER PARAMETER OF PERIPHERAL BLOOD MONONUCLEAR CELLS

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Abstract. The aim of our study was to evaluate the *in vitro* effect of vegetal antioxidants (quercetin and epigallocatechin gallate) on the membrane fluidity of human peripheral blood mononuclear cells (PBMC). 17 healthy subjects, 40 to 55 years old, were selected for this purpose. The protocol was approved by the local ethics committee and the informed consent of the patients was obtained. From *à jeun* blood samples drawn from the patients we isolated the PBMCs and incubated them, at room temperature, for 20 minutes with the above mentioned antioxidants. We evaluated the fluorescence anisotropy (*r*) and lipid order parameter (*S*) using the fluorescent TMA-DPH probe in a steady state fluorescence polarization experiment. The *in vitro* effect of flavonoids seems to result in an increase of the membrane fluidity (more than 50% of the subjects presented the effect).

Key words: flavonoids, epigallocatechin gallate, quercetin, fluorescence anisotropy, lipid order parameter, membrane fluidity.

INTRODUCTION

Flavonoids, or bioflavonoids, are a group of polyphenolic substances which are present in most plants. The extracts from the seeds, fruit skin, leaves, bark or flowers of certain plants have been reported by many authors as having antibacterial, anti-inflammatory, antiallergenic, antimutagenic, antiviral, antineoplastic, antithrombotic, and vasodilatory actions. In a study of flavonoid antioxidant activity in the aqueous phase, epicatechin gallate, epigallocatechin gallate (EGCG), and quercetin (Q) scored the highest, followed by epigallocatechin, gallic acid, epicatechin, catechin, rutin, and dihydroquercetin [6].

Epigallocatechin 3-gallate (Fig. 1) is an antimutagen extracted from the green tea (*Camelia sinensis*), that acts as a 5-α-reductase inhibitor [11]. It is thought to be useful in protecting skin against UV light-damage, in the treatment of skin tumors [3, 8] and also in the HIV treatment, in a new class of drugs [2, 12].

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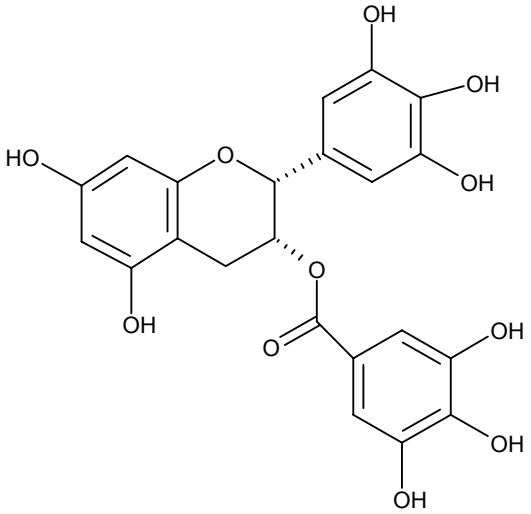

Fig. 1. The chemical structure of EGCG.

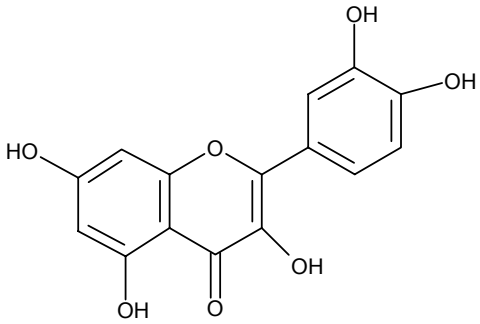

Fig. 2. The chemical structure of quercetin.

Quercetin (Fig. 2) is the aglycone form of a number of other flavonoid glycosides, such as rutin and quercitrin, found in citrus fruit, buckwheat and onions. Quercetin is the major flavonoid in our diet, and the estimated average daily dietary intake of quercetin by individuals ranges from 5–70 mg. Quercetin was reported to act as an antiinflammatory agent and to have positive effects in combating or helping to prevent cancer, prostatitis, heart and respiratory disease [1, 6, 9].

The aim of our study was to evaluate the *in vitro* effect of quercetin and epigallocatechin gallate on the fluorescence anisotropy and membrane fluidity of peripheral blood mononuclear cells (PBMC) isolated from healthy subjects, using the fluorescent probe 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene *p*-toluenesulfonate (TMA-DPH) in a steady-state fluorescence polarization experiment.

MATERIALS AND METHODS

Subjects: 17 subjects, 40 to 55 years old, were selected for the purpose. The subjects had no major illness, no previous hospital admissions, no current medication, and a subjective perception of good health as determined by health questionnaire. The general biochemical evaluation of the selected subjects (glycemia, lipids, lipoproteins, hepatic enzymes, renal function tests) was in normal range. Informed consent was obtained from each subject participating in the study.

Biological material: 5 mL of peripheral venous blood was sampled from the subjects on $EDTANa_2$ as anticoagulant.

Reagents: Hystopaque 1077 was purchased from Merck, RPMI 1640 medium was purchased from Biochrom AG, TMA-DPH was obtained from Molecular Probes.

Devices: LS50 B spectrofluorimeter (Perkin Elmer), equipped with thermostated cell holder, magnetic stirring and fluorescence polarization accessory.

From all the subjects *à jeun* venous blood samples on EDTA-Na_2 were drawn for the separation of PBMCs. The separation of the cells was performed using the density gradient method: the samples were centrifuged at 600×g for 20 minutes, at 25 °C on Hystopaque 1077. The PBMC cell ring obtained was collected washed with RPMI 1640 medium (210×g, 10 min, 4 ºC), and re-suspended in 3 mL of RPMI 1640 medium. The cells were counted in a Burker-Türk chamber, and the PBMC suspension was standardized at 10^5 cells/mL with RPMI 1640 medium.

For the membrane fluidity evaluation we assessed the TMA-DPH steady state fluorescence polarization.

We evaluated the autofluorescence of each sample of normalized PBMC suspension with excitation polarized light at 340 nm, and the emission intensities were detected at 425 nm, through another polarizer. The cell suspension was incubated with the fluorescent probe (2.5 μM TMA-DPH in the measurement cuvette) for 2 minutes at 37ºC, under continuous magnetic stirring, then the fluorescence polarization of TMA-DPH was measured again ($\lambda_{excitation}$ = 340 nm, $\lambda_{emission}$ = 425 nm).

We incubated each sample with Q (1 μM) and, respectively, EGCG (10 μM) for 20 minutes at room temperature, and then we measured again the TMA-DPH-cell membrane fluorescent properties.

Calculation of the fluorescence anisotropy (r) was performed according to equations (1) and (2):

$$r = \frac{I_{vv} - GI_{vh}}{I_{vv} + 2GI_{vh}} \quad (1)$$

$$G = \frac{I_{hv}}{I_{hh}} \quad (2)$$

where r is the anisotropy, I_{vv}, I_{vh}, I_{hv} and I_{hh} represent the emission intensities corrected for the autofluorescence signal of the cells, when the polarizers in the excitation and emission beams are oriented vertical-vertical, vertical-horizontal, horizontal-vertical and horizontal-horizontal, respectively [5].

The lipid order parameter in the polar head group (S) regions was computed following equation (3), using the limiting initial r_0 and long-time r_∞ values of the fluorescence anisotropy of TMA-DPH-membrane complex [4, 7, 10]:

$$S = \sqrt{\frac{r_\infty}{r_0}} \quad (3)$$

where $r_\infty = 1.270 \times r - 0.076$ for $0 < r < 0.28$ and $r_\infty = 1.100 \times r - 0.032$ for $0.28 < r < 0.34$ and $r_0 = 0.362$.

The membrane fluidity, f, was considered as the reciprocal of the lipid order parameter S.

Statistical analysis: results are expressed as means±standard deviation (SD). Statistical analyses were performed using the Student's t-test and differences were considered significant for $p < 0.05$.

RESULTS AND DISCUSSION

The baseline values for the biophysical parameter evaluated for the study group are presented in Table 1.

Table 1

Baseline values for the biophysical parameters evaluated for the subjects

Parameter	Study group
Fluorescence anisotropy (r)	0.28±0.03
Lipid order parameter (S)	0.88±0.06
Membrane fluidity (f)	1.14±0.08

The two tested flavonoids increased *in vitro* the membrane fluidity for 64.7% of samples treated with quercetin and for 57% of the samples treated with epigallocatechin gallate (Fig. 3).

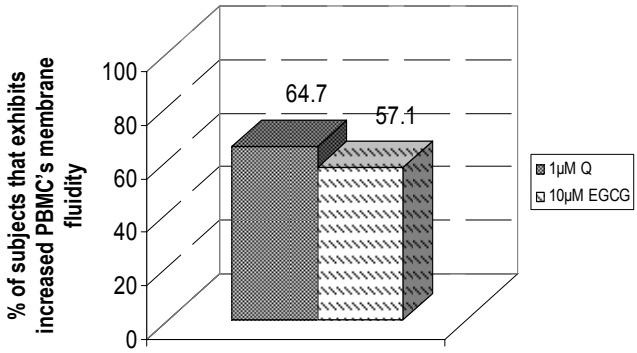

Fig. 3. The *in vitro* effect of Quercetin (Q) and EGCG treatment of PBMC in healthy subjects.

Table 2

Evaluated parameters after *in vitro* exposure of PBMC to 1 μM quercetin

Parameter	64.7 % of the study group
Fluorescence anisotropy (*r*)	0.25±0.04
Lipid order parameter (*S*)	0.80±0.09
Membrane fluidity (*f*)	1.25±0.15

Incubation with 1 μM quercetin of PBMC isolated from the subjects led to a decrease of the fluorescence anisotropy (*r*) with a corresponding increase of the membrane fluidity (*f*) and a decrease of the lipid order parameter (Table 2).

Similarly, incubation of the PBMC with 10 μM ECG determined the decrease of the *r* parameter (corresponding to a decrease of *S* and an increase of *f*), as shown in Table 3.

Table 3

Evaluated parameters after *in vitro* exposure of PBMC to 10 μM epigallocatechin gallate

Parameter	57.1 % of the study group
Fluorescence anisotropy (*r*)	0.24±0.0.02
Lipid order parameter (*S*)	0.79±0.05
Membrane fluidity (*f*)	1.27±0.09

CONCLUSIONS

The investigation of the membrane fluidity proved to be a useful and sensitive method to obtain a better insight into the mechanisms by which different compounds, including drugs, can affect the cellular functions. The study was

performed on peripheral blood mononuclear cells (PBMC) separated from healthy subjects, in a steady-state fluorescence polarization experiment, using 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene *p*-toluenesulfonate (TMA-DPH) as a fluorescent probe. The results show that the *in vitro* effect of flavonoids seems to result in an increase of the membrane fluidity (more than 50% of the subjects presented the effect). Further studies will have to look for a dose-response relationship of the effects, and also to look more carefully to the subjects that exhibit a decrease of the fluidity under the influence of the natural antioxidants in an attempt to identify the action mechanism of quercetin and epigallocatechin gallate on the cell membrane.

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