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The tea protection against the reactive oxygen species produced via the photodynamic effect induced by daylight

Martina Bancirova *

Palacký University, Department of Medical Chemistry and Biochemistry, Hnevotinska 3, 77515 Olomouc, Czech Republic

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ABSTRACT

The combination of light, photosensitizer and molecular oxygen is involved in the photodynamic effect. The life-time of ROS is extremely short and ROS can damage biological systems. The reactive oxygen species (ROS) are also produced upon excitation of the photosensitizer by visible light only. Some drinks and foods have the potential or definite antioxidant capacity to inhibit or terminate the ROS action.

Usually, *Paramecium caudatum* is used to determine the toxic effect; well known is especially the toxicity determination of the photodynamic effect. The aim of this work was to explore if the protective effect of tea against ROS produced by the different types of photosensitizer (methylene blue, eosin, fluorescein, phthalocyanines) upon the excitation by visible light only is also possible to determine on the unicellular organism *P. caudatum*, and compare the protective effect of the black and green teas against ROS with the protective effect of ascorbic acid and Trolox (a standard for the total antioxidant capacity determination).

The teas were able to prolong the *P. caudatum* life-time; the highest observed protection against the photodynamic ROS production (triggered by methylene blue) was caused by the black and green teas and was identical for both of them. The stronger protective antioxidant properties of the green tea were not observed. The pro-oxidant influence of the used antioxidants was not observed.

1. Introduction

The photodynamic effect involves the combination of light, an organic dye (photosensitizer) and molecular oxygen (Foote, 1991). Upon excitation (irradiation usually by light in the visible region), reactive oxygen species (ROS) are produced. Their life-time is extremely short, and if there a physiological acceptor to neutralize them is not present, ROS can damage the biological system.

The photochemical and photophysical principles of the photodynamic effect have been extensively studied (Dougherty et al., 1998; Hamblin & Hasan, 2004; Huang, 2005; McCaughan, 1999; Plaetzer, Krammer, Berlanda, Berr, & Kiesslich, 2009; Robertson, Hawkins Evans, & Abrahamse, 2009; Sharman, Allen, & van Lier, 1999, 2000; Wilson & Patterson, 2008). The photosensitizer is excited from the ground state S_0 to the first excited single state S_1, followed by conversion to the triplet state T_1 via intersystem crossing. The excited triplet state can react in two ways, defined as *Type I* and *Type II* mechanism. *Type I* mechanism (production of superoxide) involves electron-transfer reactions between the excited state of the photosensitizer and a substrate that is biological, a solvent or another photosensitizer, to yield free radicals and radical ions. ROS are also the important part of the oxidative stress (Maeda, 2008); it can be described as a shift in the balance between oxidants and antioxidants (Davies, 1995). The most of oxidants derive directly or indirectly from O_2. All aerobic organisms have developed more or less complex systems to neutralize ROS before their potentially harmful effect is initiated — the antioxidants. The antioxidants include, among others, vitamins (e.g. vitamin C and E), proteins and enzymes (e.g. glutathione peroxidase, catalase, superoxide dismutase) (Arrigoni & De Tullio, 2002; Burton & Ingold, 1986; Carr & Frei, 1999; Lutsenko, Carcamo, & Golde, 2002; Nagaoka, Kakiuchi, Ohara, & Mukai, 2007). The intake of food and beverages with the high antioxidant capacity (the ability of the total amount of the antioxidants to quench or scavenge ROS) is one way to prevent the oxidative stress in the organism and to maintain the balance between oxidants and antioxidants. The production of ROS by the photosensitizers is one of the examples of the oxidative stress. Photochemical generation of reactive oxygen species leads to cell death and acute changes within the tumor microenvironment.

Commercially grown teas are hybrids of two distinct ecotypes: the Assam-type (var. *assamica*) and the China-type (var. *sinensis*) (Carr & Stephens, 1992). Freshly harvested tea leaves must be processed to inactivate enzymatic oxidation for the green tea production or to control the oxidation by the leaf enzymes for the black tea production (Dufresne & Farnworth, 2001). Catechins and other polyphenols have antioxidant activities (Dufresne & Farnworth, 2001; Sarkar & Bhaduri, 2001; Wiesburger, 1999). The tea leaves produced contain a number of phenolic compounds of which four are present in significant amounts: (_)-epigallocatechin-3-gallate (EGCG); (_)-epigallocatechin (EGC); (_)-epicatechin gallate; and (_)-epicatechin. These are collectively

* Tel.: +420 585 632 304; fax: +420 585 632 302.
E-mail address: martina.bancirova@upol.cz.

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known as catechins. In addition, green tea contains (1)-gallocatechin and (1)-catechin, as well as caffeine, threophylline, threobromine and theonine (Xie, Shi, Chen, & Ho, 1993) and these green tea polyphenols can scavenge ROS with different efficacy (Afaq & Mukhtar, 2002), related to their reducing properties (Rice-Evans, 1999). They act as antioxidants *in vitro* by sequestering metal ions and by scavenging reactive oxygen and nitrogen species (Frei & Higdon, 2003; Wiseman, Balentine, & Frei, 1997). Tea also contains carbohydrates, vitamins E, K, A, low levels of B vitamins and vitamin C (in green tea only).

Tea is one of the most popular beverages with a high total antioxidant capacity. Antioxidants are primarily reducing agents prone to scavenge reactive species in one way or another. The presence of antioxidants in photodynamic reactions usually reduces the efficiency of the photodynamic therapy. Some antioxidants (e.g. ascorbic acid and α-tocopherol) added to cells at adequate concentrations may enhance the photodamage caused by the photosensitizers (Jakus & Farkas, 2005). The primary polyphenol component of green tea, EGCG, also possesses anti-carcinogenic, and anti-tumor properties, and this compound is being investigated as a cancer treatment agent when combined with chemotherapy or radiation. EGCG enhanced PDT-mediated cytotoxicity by inducing pro-apoptotic pathways and by inhibiting the expression of pro-angiogenic molecules (Raish et al., 2010).

The photodynamic effect was described on *Paramecium caudatum* solution by Raab (1900) and this *Protozoa* is still the easiest way how to determine the lethal impact of the photodynamic effect on the biological material. *Paramecium* is unicellular organisms, usually range from about 50 to 350 μm in length; therefore it is possible to observe its moving by naked eye. It is covered with minute hairlike projections called cilia. Cilia are used in locomotion and during feeding. When moving through the water, *Paramecium* follows a spiral path while rotating on the long axis. When *Paramecium* encounters an obstacle, it exhibits the so-called avoidance reaction: It backs away at an angle and starts off in a new direction. In case of the death it is immobilized, so it is easy to distinguish surviving and killed individuals.

This study is focused on the *P. caudatum* protection against ROS (produced by the photodynamic effect) by the presence of the black and green teas. The first step is to determine the suitable concentration range of the photosensitizer for the life-time observation. The second step is to determine the concentration edge of antioxidant (the concentration range of the antioxidant that does not influence the life-time of *P. caudatum* for 24 h at least). The final step is to explore the life-time prolongation in the presence of the antioxidants after the addition of the photosensitizers.

2. Materials and methods

2.1. Chemicals

Fluorescein disodium salt (FL) (Fluka, Germany, ex. wavelength 460 nm), eosin Y disodium salt (EY) (Lachema Brno, Czech Republic, dye content 85%, ex. wavelength 532 nm), phthalocyanine tetrasulphonate ($CuPcS_4$) (Sigma-Aldrich, Germany, dye content 85%, ex. wavelength 670 nm), methylene blue (MB) (Sigma-Aldrich, Germany, dye content 82%, ex. wavelength nm), eosin B (EB) (Sigma-Aldrich, Germany, dye content 90%, ex. wavelength 514 nm), ascorbic acid (AA) (Sigma-Aldrich, Germany, 99%) Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich, Germany).

2.2. Tea infusion

The stock solution of tea was prepared from 2 g of dry leaves (Earl Grey — black tea- and Gunpowder — green tea) and 100 ml of boiling distilled water (the samples and the way of preparation were identical as in the previous paper — Bancirova, 2010). After cooling and filtration, 1 ml of the tea infusion was added in solution of *P. caudatum*. The content of individual antioxidants was not determinate. The ability to protect *P. caudatum* against ROS produced by the photodynamic effect was compared with total antioxidant capacity of the tea samples.

2.3. P. caudatum life-time determination

The experiments were done in Petri dishes with *P. caudatum* aqueous solution (total volume of 10 ml, the density about 25 individuals in 1 ml) on the overhead projector (Quantum 2521, NOBO). The aqueous solution enables the free movement of *P. caudatum* in order to distinguish living (moving) and dead (immobilized) individuals of *P. caudatum*. The overhead projection replaced the microscope to make possible observation of the *P. caudatum* movement. The photodynamic effect was triggered by day light only (the sunshine did not entry the laboratory). The concentration ranges of the photosensitizer were based on our previous observations (Bancirova, 1997). There were three Petri dishes to compare: A — containing *P. caudatum* and photosensitizer, B — containing *P. caudatum* and antioxidant, C — containing *P. caudatum*, photosensitizer and antioxidant. The tea dilution was 10 fold, the final concentration of ascorbic acid was 0.57 mM and the final concentration of Trolox was 50 μM (all concentrations of the antioxidants and their mixture were tested for 24 h without killing effect, data — zero killing effect — are not shown). The life-time was determined after addition of all components of the measurement arrangements (the sample of the antioxidant was the first one, the photosensitizers was the last one). The life-time is the time period during which the movement of *P. caudatum* was observed. The end of the life-time means the solution without moving *P. caudatum* (so it could be supposed that all individuals of *P. caudatum* are dead). The figures show the dependence of the *P. caudatum* life-time on the photosensitizer concentration (see Fig. 1a,b) and the dependences of the *P. caudatum* life-time prolongation on the photosensitizer concentration in the presence of the antioxidant (see Figs. 2–6). The observation of the *P. caudatum* movement was done by the naked eye or during the inching overhead project in the regular intervals to project — enlarge them (interval was based on the preliminary determination of the life-time). The potential influence of the higher temperature on the overhead projector surface on *P. caudatum* life-time was checked by the simultaneous presence of one sample in the Petri dish included *P. caudatum* solution only for the whole time period of the measurement. It means that the blank control of *P. caudatum* was placed on the overhead projector surface at least 12 h without changes in *P. caudatum* behavior (they have shown their typical movement; in contrary to their behavior immediately after the addition of the chemicals when they have clustered).

Values are means ± SD of three independent experiments. Statistical significance was determined using Student's unpaired two-tailed *t*-test. *P* value less than 0.05 was statistically significant.

3. Results and discussion

The photodynamic effect was described more than 100 years ago just on *P. caudatum* solution (Raab, 1900). This way how to prove the killing effect of the photosensitisers on the biological material is still suitable.

The experiments have included three types of Petri dishes: A — containing *P. caudatum* and photosensitizer, B — containing *P. caudatum* and antioxidant, C — containing *P. caudatum*, photosensitizer and antioxidant.

As the first step, it was necessary to determine the concentration range of the photosensitizer killing effect. The selected photosensitizers — eosin B, eosin Y, methylene blue and fluorescein — are well known used to induce the photodynamic effect. Their abilities to kill *P. caudatum* were compared with the commercially available phthalocyanines. See Fig. 1a,b (the data shown in the graphs are the average values of three measurements).