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Short communication

Differences in the hydroxylation pattern of flavonoids alter their chemoprotective effect against direct- and indirect-acting mutagens

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ABSTRACT

The antimutagenicity of ten flavonoids, differing in their hydroxylation patterns against direct-acting and indirect-acting mutagens, namely 4-nitro-*o*-phenylenediamine, sodium azide, mitomycin C, benzo[*a*]pyrene, aflatoxin B_1 and 2-aminofluorene, were compared with the aim of investigating how the hydroxyl groups in their structures govern the biological activity of flavonoids, by the Ames test, with *Salmonella typhimurium* strains TA98, TA100 and TA102. The flavonoids tested were: quercetin, kaempferol, luteolin, fisetin, chrysin, galangin, flavone, 3-hydroxyflavone, 5-hydroxyflavone and 7-hydroxyflavone. In these tests, all compounds were shown to be antimutagenic in more than one strain and various mechanisms of action were demonstrated. The results suggested that the number and position of hydroxyl groups may increase or decrease the protective effect, depending on the type and concentration of flavonoids and mutagen used. These studies contribute to clarifying the mechanisms by which these flavonoids act in protecting DNA from damage. This is required before they can be widely used.

1. Introduction

Consumption of fruit and vegetables plays a vital role in the natural prevention and treatment of various diseases. They are known to be rich in polyphenolic compounds, particularly flavonoids, compounds that have been attracting interest because of their significant bioactivity. Health benefits that have been reported for flavonoids include antioxidant, antithrombotic, antidiabetic, anticancer, and vasodilatory activities (Mäkynen et al., 2013).

In an attempt to clarify the mechanisms of action, in particular those involved in protection against DNA damage, this study demonstrates the antimutagenic potential of ten flavonoids (Fig. 1) against the mutagenicity of a variety of direct- and indirect-acting mutagens, namely 4-nitro-*o*-phenylenediamine (NPD), sodium azide (SA), mitomycin C (MMC), benzo[*a*]pyrene (B[*a*]P), aflatoxin B_1 (AFB_1) and 2-aminofluorene (2-AF), by the Ames test, showing them to be promising pharmaceutical and nutraceutical compounds.

2. Materials and methods

2.1. Chemicals and culture media

Quercetin, kaempferol, fisetin, luteolin, flavone, 3-hydroxyflavone, 5-hydroxyflavone, 7-hydroxyflavone, chrysin, galangin (minimum purity of flavonoids 90–98% by HPLC), dimethyl sulfoxide (DMSO), nicotinamide adenine dinucleotide phosphate sodium salt (NADP), D-glucose-6-phosphate disodium salt, magnesium chloride, L-histidine monohydrate, D-biotin, NPD, SA, MMC, 2-AF, B[*a*]P and AFB_1 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Oxoid Nutrient Broth No. 2 (Oxoid, England) was used as bacterial media. D-Glucose, magnesium sulfate, citric acid monohydrate, anhydrous dibasic potassium phosphate, sodium ammonium phosphate, monobasic sodium phosphate, dibasic sodium phosphate and sodium chloride were purchased from Merck (Whitehouse Station, NJ, USA).

2.2. Antimutagenicity assay

Antimutagenic activity was evaluated by the *Salmonella*/microsome assay, using the *Salmonella typhimurium* tester strains TA98, TA100, and TA102, kindly provided by Dr. B.N. Ames (Berkeley, CA, USA). The flavonoids were combined with known mutagens in tests, with and without metabolic activation. The strains from frozen cultures were grown overnight for 12–14 h in Oxoid Nutrient

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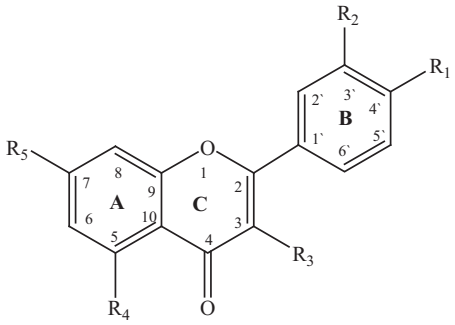

	R_1	R_2	R_3	R_4	R_5
Quercetin	OH	OH	OH	OH	OH
Kaempferol	OH	H	OH	OH	OH
Fisetin	OH	OH	OH	H	OH
Luteolin	OH	OH	H	OH	OH
Chrysin	H	H	H	OH	OH
Galangin	H	H	OH	OH	OH
Flavone	H	H	H	H	H
3- hydroxyflavone	H	H	OH	H	H
5- hydroxyflavone	H	H	H	OH	H
7- hydroxyflavone	H	H	H	H	OH

Fig. 1. Molecular skeleton of flavonoids.

Broth No. 2. The metabolic activation mixture (S9 fraction), prepared from livers of Sprague–Dawley rats treated with the polychlorinated biphenyl mixture Aroclor 1254 (500 mg/kg), was purchased from Molecular Toxicology Inc. (Boone, NC, USA) and freshly prepared before each test. The metabolic activation system consisted of 4% S9 fraction, 1% 0.4M $MgCl_2$, 1% 1.65M KCl, 0.5% 1M D-glucose-6-phosphate disodium, 4% 0.1M NADP, 50% 0.2M phosphate buffer and 39.5% sterile distilled water (Maron & Ames, 1983).

Five different doses of the test compounds were assayed. All of them were dissolved in DMSO. In these tests, the direct-acting mutagens were used at 10.0 μg/plate of NPD (for *S. typhimurium* TA98), 1.25 μg/plate of SA (for *S. typhimurium* TA100) and 0.5 μg/plate of MMC (for *S. typhimurium* TA102) and the indirect-acting mutagens were used at 1.0 μg/plate of B[*a*]P (for *S. typhimurium* TA98), 0.5 μg/plate of AFB_1 (for *S. typhimurium* TA100) and 10 μg/plate of 2-AF (for *S. typhimurium* TA102). The various concentrations of flavonoids plus mutagens to be tested were added to 0.5 ml of 0.2M phosphate buffer, or to 0.5 ml of S9 mixture, with 0.1 ml of bacterial culture and then incubated at 37 °C for 20–30 min. Next, 2 ml of top agar were added and the mixture poured onto a plate containing minimal agar. The plates were incubated at 37 °C for 48 h and the His^+ revertant colonies were counted manually. All experiments were analyzed in triplicate.

The antimutagenicity results were expressed as percent inhibition (ability of a compound to inhibit the action of the known mutagen). This was calculated as follows:

$$\text{Inhibition}(\%) = 100 - [(T/M) \times 100]$$

where *T* is the number of revertant colonies on the plate containing mutagen and compound and *M* is the number of revertant colonies on the plate containing only the mutagen (Loh, Er, & Chen, 2009).

No antimutagenic effect was recorded when inhibition was lower than 25%, a moderate effect for a value between 25% and 40%, and strong antimutagenicity for values greater than 40% (Lira et al., 2008; Resende et al., 2012a,b).

Cell viability was also determined for each antimutagenesis experiment, to assess the potential bactericidal effect of the mutagens. A substance was considered cytotoxic when the bacterial survival was less than 60% of that observed for the negative control (Lira et al., 2008; Resende et al., 2012a,b).

3. Results and discussion

In the present study, the antimutagenic activities of the flavonoids quercetin, kaempferol, luteolin, fisetin, chrysin, galangin, flavone, 3-hydroxyflavone, 5-hydroxyflavone and 7-hydroxyflavone were assayed by the Ames test and are presented in Table 1, expressed as percent inhibition, in bacterial strains TA98, TA100 and TA102 in combination with direct and indirect mutagens. These compounds differ from one another only by the number and position of the hydroxyl groups present in the molecule. All compounds exhibited a protective effect against the mutagenic action of direct- and indirect-acting mutagens. They showed antimutagenic potential in more than one bacterial strain and exerted their effects by various mechanisms of action. Thus, the number and position of hydroxyl groups may increase or decrease the effect of protection, depending on the type and concentration of the flavonoid used.

In a previous study, we observed that quercetin was itself mutagenic and induces frameshift mutations (TA98), substitution of base pairs (TA100) and oxidative damage (TA102) (Resende et al., 2012a,b). Usually, however, the protection induced by quercetin