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The interaction of flavonoids with mitochondria: effects on energetic processes

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Abstract

The study addressed aspects of energetics of isolated rat liver mitochondria exposed to the flavonoids quercetin, taxifolin, catechin and galangin, taking into account influences of the 2,3 double bond/3-OH group and 4-oxo function on the C-ring, and *o*-di-OH on the B-ring of their structures, as well as mitochondrial mechanisms potentially involved in cell necrosis and apoptosis. The major findings/hypothesis, were: The 2,3 double bond/3-OH group in conjugation with the 4-oxo function on the C-ring in the flavonoid structure seems favour the interaction of these compounds with the mitochondrial membrane, decreasing its fluidity either inhibiting the respiratory chain of mitochondria or causing uncoupling; while the *o*-di-OH on the B-ring seems favour the respiratory chain inhibition, the absence of this structure seems favour the uncoupling activity. The flavonoids not affecting the respiration of mitochondria, induced MPT. The ability of flavonoids to induce the release of mitochondria-accumulated Ca^{2+} correlated well with their ability to affect mitochondrial respiration on the one hand, and their inability to induce MPT, on the other. The flavonoids causing substantial respiratory chain inhibition or mitochondrial uncoupling, quercetin and galangin, respectively, also decreased the mitochondrial ATP levels, thus suggesting an apparent higher potential for necrosis induction in relation to the flavonoids inducing MPT, taxifolin and cathechin, which did not decrease significantly the ATP levels, rather suggesting an apparent higher potential for apoptosis induction.

Keywords: Flavonoids; Mitochondria; Mitochondrial energetics; Mitochondrial permeability transition; Permeability transition pores; Mitochondrial Ca^{2+} efflux; Necrosis; Apoptosis

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; CsA, cyclosporin A; EGTA, ethylene glycol bis(-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, *N*-(2-hydroxyethyl) piperazine-*N*′-(2-ethanesulfonic acid); MPT, mitochondria permeability transition; PTP, permeability transition pores; TMA-DPH, 1,6-diphenyl-1,3,5-hexatriene-4-trimethylammonium trimethylammonim tosylate; TRIS, tris(hydroxymethyl)-aminomethane

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1. Introduction

The proton motive force generated by respiratory chain in the inner mitochondrial membrane drives the oxidative phosphorylation process, which supplies the majority of the ATP required by cell. In the presence of high Ca^{2+} levels, the proton motive force leads to the cation accumulation into mitochondria, mainly via an electrogenic transporter–uniporter. On the other hand, Ca^{2+} is released from organelle via either Na^{+}-dependent or Na^{+}-independent efflux mechanisms, as well as via mitochondrial permeability transition (MPT) [1,2]. MPT is a Ca^{2+}-dependent cyclosporin-A (CsA)-sensitive process mediated by the opening of membrane channels, named permeability transition pores (PTP), which causes mitochondrial swelling/inner transmembrane potential dissipation [3–7]. Both cell Ca^{2+} compartmentalization [8–10] and MPT [11–13], have been widely implicated as playing important roles in cell death by either necrosis or apoptosis. MPT, in particular, modulates apoptosis by releasing from mitochondria apoptogenic factors such as cytochrome *c*, a respiratory chain component.

Flavonoids are benzo-γ-pyrone derivatives widely distributed in the vegetal kingdom, possessing well-recognised antioxidant and prooxidant properties. Their fundamental structure comprises two benzene rings (A and B) linked through a heterocyclic pyran or pyrone (with a double bond) ring (C) in the middle. In contrast with the structural features favouring the antioxidant activity of these compounds [14], those favouring their prooxidant activity have not yet been established. In mitochondria, prooxidants and antioxidants are usually potential MPT inducers or inhibitors, respectively.

In a previous study [15] we demonstrated that depending on their antioxidant properties a series of naturally occurring flavonoids protect from the onset of MPT, being therefore potentially capable of acting as necrosis and apoptosis cytoprotecting agents, although recent reports [16,17] have shown that some flavonoids may promote apoptosis in association with prooxidant/MPT inducing activities. In this context, disruption of apoptosis by cell has been associated with tumor initiation, progression and metastasis [18,19], and flavonoids have been considered as promising anticancer agents [20]. In the present study we address aspects of energetics of isolated rat liver mitochondria exposed to the flavonoids quercetin, taxifolin, catechin and galangin (Fig. 1), taking into account influences of the 2,3 double bond/3-OH group and 4-oxo function on the C-ring, and *o*-di-OH on the B-ring of their structures, as well as mitochondrial mechanisms potentially involved in cell necrosis and apoptosis.

2. Materials and methods

2.1. Chemicals

Flavonoids were purchased from Sigma/Aldrich. All other reagents were of the highest commercially available grade. The amounts of dimethyl sulfoxide required to solubilize the flavonoids had no effect on the assays. All stock solutions were prepared using glass-distilled deionized water (2 μM of contaminant Ca^{2+}).

2.2. Isolation of rat liver mitochondria

Mitochondria were isolated by standard differential centrifugation [21]. Male Wistar rats weighing approximately 200 g were sacrificed by cervical dislocation; livers (10–15 g) were immediately removed, sliced in 50 ml of medium containing 250 mM sucrose, 1 mM EGTA and 10 mM HEPES–KOH, pH 7.2, and homogenized three times for 15 s at 1 min intervals with a Potter–Elvehjem homogenizer. Homogenates were centrifuged at $580 \times g$ for 5 min and the resulting supernatant was further centrifuged at $10300 \times g$ for 10 min. Pellets were suspended in 10 ml of medium containing 250 mM sucrose, 0.3 mM EGTA and 10 mM HEPES–KOH, pH 7.2, and centrifuged at $3400 \times g$ for 15 min. The final mitochondrial pellet was suspended in 1 ml of medium containing 250 mM sucrose and 10 mM HEPES–KOH, pH 7.2, and used within 3 h. Mitochondrial protein content was determined by the biuret reaction.

2.3. Standard incubation procedure

Mitochondria energized with 5 mM potassium succinate (+2.5 μM rotenone) in a standard incubation medium containing 125 mM sucrose, 65 mM KCl and 10 mM HEPES–KOH, pH 7.4, were incubated at 30 °C with different flavonoids concentrations.