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Comparison of cell based in vitro assays as predictors for in vivo bioactivity of polyphenol compounds

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A range of different in vitro assays are currently being used to evaluate antioxidant activity of polyphenol preparations, and results are being extrapolated to predict in vivo bioactivity. However, those assays are mostly based on the structural features of polyphenols (such as hydroxyl substituents, 2,3-double bond and 4-oxo-group) and reflect chemical reactivity rather the potency of polyphenols to elicit a cellular response. The aim of this study therefore was to compare the antioxidant activity of selected flavonols with markers of oxidative stress in isolated erythrocytes and mitochondria fractions as well as in cultivated cells.

Quercetin, isorhamnetin and kaempferol were assayed for their antioxidant activity (FRAP assay) using standard protocols. Isolated human erythrocytes[1] were incubated with flavonol compounds (25μM) and the plasma membrane redox system (PMRS), indicating changes in erythrocyte redox levels, was determined. Mitochondria isolated from rat liver tissue[2] were used to quantify iron sulfate induced lipid peroxidation (LPO) as well as hydrogen peroxide (H_20_2) generation (Amplex Red Assay) following flavonol incubation (25μM and 5μM, respectively). Results were compared with the capacity of flavonols (25μM) to inhibit H_20_2 induced mitochondrial DNA damage[3] in human fibroblast cells (real time qPCR assay).

Results of FRAP and erythrocyte PMRS assays indicate highest antioxidant potency for quercetin in comparison to isorhamnetin and kaempferol whereas mitochondria based LPO shows similar values for quercetin and isorhamnetin; both being able to inhibit mitochondria LPO by over 80 % (at 25μM). Similarly, mtDNA damage is attenuated by quercetin and isorhamnetin by around 50 % whereas kaempferol induced reduction counts only around 25 %. Hydrogen peroxide production in mitochondria following flavonol incubation is strongly inhibited by isorhamnetin (94·6 %) and to a much lesser extent by quercetin (31·6 %) and kaempferol (13·4 %). Dose dependent relationships can be found for all parameters (data not shown).

	FRAP (μM trolox equiv.)		Erythroyte PMRS (% control)		Mitochondria LPO inhibition (% control)		Mitochondria H_20_2 inhibition (% control)		mtDNA damage inhibition (% control)	
Compound	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Quercetin	484	18·2	248[a]	0·83	80·2[a]	0·36	31·6[a]	8·9	48·2[a]	4·1
Isorhamnetin	230	17·7	151[b]	10·5	89·6[a]	1·3	93·6[b]	2·9	52·5[a]	3·4
Kaempferol	199	20·0	176[b]	17·0	53·1[b]	3·2	13·4[c]	0·1	25·4[b]	5·9

Data are mean of three measurements in duplicate. * Indicates signficant difference to control ($p < 0·05$)

In summary, PMRS seems to reflect values of common antioxidant assays such as obtained by FRAP, whereas the inhibition of LPO in isolated mitochondria by the test compounds more closely resembles cellular flavonol effects and might therefore have greater value for evaluations of antioxidant capacity of polyphenol compounds.

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