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Is thymoquinone an antioxidant?

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Background

Thymoquinone is one of the active ingredients of black cumin (*Nigella sativa* L.) essential oil possessing anti-inflammatory, antineoplastic, neuro- and hepatoprotective properties. Some of these properties were attributed to an antioxidant activity of thymoquinone, which seems to be unlikely from its structure. Because the lipophilic thymoquinone exhibits a structural similarity with the natural mitochondrial electron carrier, ubiquinone, it was of interest whether the suggested antioxidant effect of thymoquinone in cells can be explained by its interaction with the mitochondrial respiratory chain.

Materials and methods

Antioxidant activities were determined spectrophotometrically by means of the 2,2-diphenyl-1-picrylhydrazyl-radical (DPPH$^{\bullet}$) assay (516 nm) as well as by the $O_2^{\bullet -}$-dependent xanthine / xanthine oxidase / cytochrome c assay (550 nm). NADH and succinate:thymoquinone oxidoreductase activities of KCN-blocked (1 mM) submitochondrial particles (0.01 mg/ml) from bovine heart were determined at 340 and 257.5 nm, respectively.

Results

With the DPPH$^{\bullet}$ assay thymoquinone was shown to be hardly antioxidative. In contrast, thymohydroquinone was even more active than the vitamin E analogon pentamethylchromanol (rate constants for the reaction with DPPH$^{\bullet}$: 283.7 ± 1.9 M^{-1} s^{-1} (3) vs. 236.2 ± 4.7 M^{-1} s^{-1} (6); data are means ± SEM (n). The cytochrome c system turned out to be unsuitable for the evaluation of the antioxidant activity of thymoquinone. Thymoquinone concentration-dependently (20–1000 μM) stimulated the NADH (150 μM) oxidation of submitochondrial particles to V_{max} values of around 400 nmol per min per mg protein. K_M values were 77.4 μM for thymoquinone and 6.4 μM for NADH. The thymoquinone-stimulated NADH oxidation was sensitive to inhibitors of the mitochondrial electron transfer (90% inhibition with 0.2 μM rotenone, 49% inhibition with 1 μM antimycin A, 82% inhibition with 1 μM antimycin A + 1 μM myxothiazol; 100 μM thymoquinone). In addition to its stimulatory effect on NADH:quinone oxidoreductase, thymoquinone (50 μM) was reduced to its hydroquinone by rotenone- (0.2 μM) and KCN-inhibited submitochondrial particles when succinate (10 mM) was used as substrate. This reduction was sensitive to antimycin A and myxothiazol, inhibitors of mitochondrial complex III.

Conclusions

Thymoquinone (oxidized form) possesses a very low antioxidant activity while its reduced form (thymohydroquinone) exerts a high radical-scavenging capacity, comparable to that of pentamethylchromanol, a short-chain tocopherol analogon. We assume that the mitochondrial respiratory chain is significant for the antioxidant properties of thymoquinone in the cell by converting the administered thymoquinone into its hydroquinone.

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