Redox Properties of Novel Antioxidant 5,8-Dihydroxycoumarin: Implications for its Prooxidant Cytotoxicity

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Z. Naturforsch. **60 c**, 849–854 (2005); received June 16/30, 2005

The aim of this work was to characterize the redox properties of the new antioxidant 5,8-dihydroxycoumarin (5,8-DHC), isolated from sweet grass (*Hierochloë odorata* L.), and to determine its impact on its cytotoxic action. Reversible electrochemical oxidation of 5,8-DHC at pH 7.0 was characterized by the midpoint potential ($E_{p/2}$) of 0.23 V vs. the normal hydrogen electrode. 5,8-DHC was slowly autoxidized at pH 7.0, and it was active as a substrate for peroxidase (POD, EC 1.11.1.7) and tyrosinase (TYR, EC 1.14.18.1). Oxidation of 5,8-DHC by POD/H_2O_2 yielded the product(s) which reacted with reduced glutathione and supported the oxidation of NADPH by ferredoxin:$NADP^+$ reductase (FNR, EC 1.18.1.2) and NAD(P)H:quinone oxidoreductase (NQO1, DT-diaphorase, EC 1.6.99.2). The concentration of 5,8-DHC for 50% survival of bovine leukemia virus-transformed lamb kidney fibroblasts (line FLK) during a 24-h incubation was (60 ± 5.5) μM. Cytotoxicity of 5,8-DHC was decreased by desferrioxamine, catalase, the antioxidant *N*,*N*'-diphenyl-*p*-phenylene diamine, and potentiated by 1,3-bis-(2-chloroethyl)-1-nitrosourea and dicumarol, an inhibitor of NQO1. This shows that 5,8-DHC possesses the oxidative stress-type cytotoxicity, evidently due to the action of quinodal oxidation product(s). The protective effect of isoniazide, an inhibitor of cytochrome P-450 2E1, points to hydroxylation of 5,8-DHC as additional toxification route, whereas the potentiating effect of 3,5-dinitrocatechol, an inhibitor of catechol-*o*-methyltransferase (COMT, EC 2.1.1.6), points to the *o*-methylation of hydroxylation products as the detoxification route.

Key words: Hydroxycoumarins, Antioxidants, *Hierochloë odorata* L.