EFFECTS OF GLUCORAPHANIN ON XENOBIOTIC METABOLIZING ENZYMES AND FREE RADICAL GENERATION IN RAT LIVER

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Studies linking fruit and vegetables intake to lower cancer risk have led to the suggestion that eating broccoli, or other *Brassicaceae*, will help an individual to lead a long and healthy life. There has also been a strong belief that mass administration of isolated natural dietary (from plants) constituents or extracts can help reduce the incidence of cancer. While the exact mechanism is not known, it is believed that the protective effect of cruciferae is due to the up-regulation of post-oxidative enzymes and/or the down-regulation of carcinogen bioactivating enzymes by phytoalexins such as sulforaphane, the byproduct of the natural constituent glucoraphanin. In practice, sulforaphane is released from its thioglucoside precursor, glucoraphanin, by myrosinase hydrolysis.
Having developed a method to produce glucoraphanin, in this investigation the validity of the proposed theory was verified at molecular level, using an animal model.
Male Sprague-Dawley rats (aged 6-7 weeks, 150±10 g) maintained on a standard laboratory diet received by mouth daily 24 or 48 mg per kg body weight of glucoraphanin (synthesized through chemoselective oxidation of glucoerucin, isolate from ripe seed of *Eruca sativa*) for four consecutive days; controls received saline only. Rats were fasted 16 h before being killed humanely in accordance with approved Home Office procedures appropriated for the species. Ten rats were used in each group; liver microsomes were prepared and immediately tested for CYP content and various monooxygenases such as ethoxyresorufin O-deethylase (CYP1A1-linked), methoxyresorufin O-demethylase (CYP1A2) and testosterone hydroxylase (CYP2B1, CYP3A1/2) as well as for the phase-II markers glutathione S-transferase (toward 1-chloro-2,4-dinitrobenzene and 1,2-epoxy-3-[p-nitrophenoxy]propane) and UDP-glucuronosyl transferase.
Contrarily to that expected, it was found that, in rat liver, glucoraphanin slightly affects phase-II "detoxifying" enzymes, but powerfully induces phase-I bioactivating enzymes such as CYP1A1 (up to ~8.8-fold, activating polycyclic aromatic hydrocarbons, PHAs), CYP3A1/2 (~4.4-fold, activating nitropyrenes, aflatoxins, PHAs) and CYP1A2-CYP2B1/2 (~5.6-fold, activating dioxins and olefins/halogenated hydrocarbons, respectively) associated mixed function monooxygenases. All increases were statistically highly significant as compared with controls ($P<0.01$, Wilcoxon).
Due to the recognized role of oxygen centred free radicals as a factor that advances the incidence of human cancer, the electron paramagnetic resonance (EPR) spectroscopy coupled to a spin-trapping technique was used to evaluate the precise contribution of CYPs induced by glucoraphanin on free radical production. A significant association between CYP induction and free radical over-generation in liver was observed.
These results indicate that glucoraphanin, the natural precursor of the supposedly chemopreventive agent of cruciferae, may have co-carcinogenic properties by boosting bioactivating mechanisms and generating an oxidative stress.