

Inactivation of thyroid peroxidase by genistein and daidzein in vitro and in vivo: mechanism for anti-thyroid activity of soy.

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ABSTRACT

The association between soybean consumption and goiter in animals and humans has a long history. Current evidence for the beneficial effects of soy requires a full understanding of potential adverse effects as well. We previously identified the isoflavones, genistein and daidzein, as the only anti-thyroid constituents present in soy (ref. 3). Genistein and daidzein caused time- and H₂O₂-dependent irreversible inactivation of bovine lactoperoxidase (LPO) and porcine thyroid peroxidase (TPO), the enzyme that synthesizes thyroid hormones. The inactivation kinetics were consistent with a suicide mechanism and the apparent dissociation constants and partition ratios for genistein and daidzein were 0.2 and 0.5 μM, and 1 and 3, respectively. Radiolabeled genistein became covalently bonded to LPO concomitant to loss of enzyme activity (approximately 4 moles genistein/mole of enzyme inactivated). Minimal effects are seen on the prosthetic heme. These data are consistent with potent mechanism-based inactivation of LPO and TPO in which radical products derived from oxidation of genistein and daidzein inactivate the peroxidases by covalent binding to critical amino acid residues. Anti-thyroid effects were also investigated in Sprague-Dawley rats fed a diet supplemented with genistein aglycone (0-500 μg/g chow) through postnatal day 140 following exposure to genistein *in utero* and maternal milk. At sacrifice, blood and thyroid glands from male and female rats were snap frozen. Both aglycone and conjugated forms of genistein were quantified in blood and thyroid glands using isotope dilution LC-electrospray MS. A dose-dependent increase was observed in both compartments. In blood, the aglycone content was 1-2% of total genistein and in thyroid it was 18-28%. Furthermore, TPO activity was depressed in a dose-dependent manner in the thyroids of both male and female rats. These data show that genistein is present in the thyroid at concentrations equivalent to those causing enzyme inactivation *in vitro* and that this is sufficient to inactivate rat TPO *in vivo*. The consequences of TPO inactivation await confirmation by histopathological evaluation, but the results are consistent with the reported goitrogenic effects of soy and underscore the potential for thyroid toxicity from isoflavones at the levels actually observed in human blood.