RESIDUE TRYPsin INHIBITOR: DATA NEEDS FOR RISK ASSESSMENT

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ABSTRACT

Trypsin inhibitor (TI) occurs naturally in many foods from plants, notably soybean protein products. Heat treatment inactivates TI and improves nutritional quality, but residual TI activity of 5 to 20% remains after typical commercial treatments. Chronic feeding of TI or products that contain TI can inhibit trypsin and chymotrypsin, stimulate their secretion, cause hypertrophy and hyperplasia of the pancreas, and lead to adenomas and carcinomas of the exocrine pancreas. In the rat, TI promotes pancreatic carcinogenesis initiated by azaserine. Data needed for possible risk assessment on TI would include 2-year bioassays from animals treated with TI and fed diets carefully controlled for type and amount of fat (which also promotes pancreatic carcinogenesis). The effects of TI on protein nutrition would have to be considered when identifying the maximum tolerated dose. Major reductions in human dietary TI exposure may not be feasible because of the multiple sources of TI, the substantial promotion by other factors such as fat, and the adverse effects of excessive heat on food products. For risk assessment of TI in a particular food, other promotors and the feasibility of decreasing TI intake must be considered.

TRYPSIN INHIBITORS IN FOODS

Natural protease inhibitors are a diverse group of proteins with wide distribution in plants commonly used as human foods. Within the broad category of natural protease inhibitors are trypsin inhibitors (TIs), many of which also inhibit chymotrypsin and other proteinases. TIs occur in many common food plants, including soybeans, other beans and seeds, and potatoes (Rackis and Gumbmann, 1981; Wilson, 1981). Certain nonprotein compounds of low molecular weight are effective inhibitors of trypsin activity (Müller, et al., 1988).

The feeding of heat-treated soybeans promotes the growth of rats (Liener, 1986). Because raw soybeans contain heat-labile TIs, it was assumed at first that their antinutritive effect was caused by the inhibition of protein digestion and that heat treatment would eradicate this effect. These assumptions, however, were not consistent with the subsequent observation that the addition of TI to free amino acid diets also inhibited the growth of rats.
Feeding raw soybeans or TI concentrates to rats led to hypertrophy and hyperplasia of the pancreas. The increased output of pancreatic enzymes is quantitatively sufficient to explain the antinutritive effect of TI on protein nutrition and account for the growth depression caused by the fecal loss of endogenous nitrogen as digestive enzymes, which substantially drains the protein supply. The pancreatic hypertrophy and hyperplasia, however, produced neoplastic effects which were not directly related to protein nutrition.

Soybeans have two major types of TIs: the Kunitz inhibitor (about 20,000 daltons) and the extensively disulfide cross-linked Bowman-Birk inhibitor (about 8,000 daltons). Although heat treatment improves the nutritional quality of soybean products by destroying TI activity, standard heat-processing methods often leave 5 to 20% of the original TI content (Rackis and Gumbmann, 1981). Enzyme activity assays do not distinguish between the two types of TIs. Enzyme-linked immunosorbent assay (ELISA) methods are being developed (Brandon et al., 1988) for rapid quantitation of the amounts of the two types of TIs in raw and processed soy products. The importance of the proportion of the two inhibitors on possible adverse effects is uncertain.

EFFECTS ON THE PANCREAS

The use of soybean products has increased in the US diet during a period in which deaths from pancreatic cancer have also increased (Mack, 1982; Roebuck, 1987). Except for the role of smoking, the etiology of human pancreatic cancer is not known, but results from animal research on TI effects on pancreatic oncogenesis suggest a causal relationship.

Ingestion of protein by the rat increases output of trypsin and chymotrypsin, which is regulated through a feedback control mechanism, probably involving cholecystokinin (CCK) (Fushiki and Iwai, 1989). Treatment of rats with dietary soybean TI causes similar increases in enzyme secretion, and continued treatment with TI is associated with hypertrophy and hyperplasia of the exocrine (acinar) cells (Roebuck, 1987; Smith, et al., 1989).

Although feeding of TI to animals for a few weeks causes hypertrophy and hyperplasia, no overt neoplastic changes occur unless the animals are also treated with a pancreatic carcinogen, such as azaserine (Liener, 1986; Roebuck, 1987). Treatment with azaserine followed by 4 to 8 weeks of dietary TI leads to atypical acinar cell foci; with continued TI treatment, these foci grow and adenomas are observed as early as 4 months, and adenomas and adenocarcinomas may be observed after about a year. In this paradigm, TI seems to be a classic promotor of azaserine-initiated pancreatic cancer. Long-term feeding of TI (1 or 2 years) without treatment with azaserine or any other initiator may result in progression of hypertrophy and atypical acinar cell foci to adenoma and carcinoma (Melmed et al., 1976; Rackis et al., 1979; McGuiness et al., 1980).

The relationship between TI and pancreatic carcinogenesis is still obscure, partly because many studies in this area probably were inherently confounded by use of full-fat soybean flour. High levels of dietary fat promote pancreatic carcinogenesis (Roebuck et al., 1981). The low molecular weight nonpolymer Camostat [N,N-dimethylcarbamoylmethyl-p-(p-guanidobenzoyloxy)phenylacetate] has a powerful trypsin inhibitory action (Fujii, 1977) and also produces CCK-8-sensitive pancreatic hypertrophy and hyperplasia (Mueller et al., 1988) similar to that produced by soybean TI (Liddle et al., 1984). These studies imply that trypsin inhibition may enhance pancreatic pathology associated with