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Letters to the Editor

Correspondence re: D. S. Swaffar *et al.*, Inhibition of the Growth of Human Pancreatic Cancer Cells by the Arginine Antimetabolite L-Canavanine. Cancer Res., *54:* 6045–6048, 1994

The ability of the ARG[1] analogue, CAV, to inhibit the growth of human pancreatic cancer cells was examined by Swaffar *et al.* (1). The authors hypothesize that ARG and CAV may be competitively incorporated into cellular proteins, where CAV incorporation results in protein degradation, ultimately leading to detrimental effects on cellular growth. However, is this incorporation the key factor in growth inhibition? An alternative hypothesis relates to the critical role played by ARG as an enzyme substrate molecule.

ARG is a substrate molecule for a class of enzymes referred to as NOS. These enzymes catalyze the conversion of ARG to NO and L-citrulline. NOS may play a multidimensional role in facilitating the growth of transformed cells. In transformed C6 glial cells, NO synthesis activates a cyclic GMP-dependent protein kinase through accumulation of cyclic GMP (2). This pathway led to significant increases in DNA synthesis and cellular proliferation (2). The inducible form of NOS has been shown to be involved in cellular iron homeostasis (3). The generation of angiogenic activity by human monocytes has been shown to be dependent on NOS activity (4). NOS mediates the vasodilatory action of tumor microvasculature, which can lead to increased blood flow during high NO expression (5). NO release has a further effect in promoting blood vessel permeability, allowing the influx of plasma proteins (6). CAV is a known inhibitor of NOS with an affinity that varies depending on the NOS isoform (7).

The class of arginase enzymes may also play an important role. Arginase is the first enzyme in the urea cycle that converts ARG to urea and L-ornithine. This enzyme has been implicated in the active stimulation of malignant growth (8). Polyamines, which have been shown to be essential molecules in the development and proliferation of neoplastic growth, require L-ornithine as a precursor (9). It has been demonstrated that CAV can reduce arginase activity *in vivo* (10). Its presence may result in competitive binding between NOS and arginase for this substrate, which in turn may be under tumor-induced cytokine control. The dramatic reversal that was observed upon the addition of ARG could, therefore, be explained by the enzymatic disinhibition that would occur in the presence of ARG.

The growth and development of solid tumors has often been compared to the cellular activities that occur during wound repair (11). In the initial stages of wound healing, NOS activity is significantly higher than that of arginase; however, after this initial stage (approximately 3 days) NOS activity declines, while arginase activity predominates (12). The key to malignant cell proliferation may arise from a temporal balance of these two mechanisms. CAV, which has known inhibitory effects on both enzyme classes, may repress malignant growth through the disruption of this pathway. The potential for a broader application of CAV in the treatment of malignancy may, therefore, be limited because of its inhibitory effects on enzymes with such varied physiological activities.

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[1] The abbreviations used are: ARG, L-arginine; CAV, L-canavanine; NOS, nitric oxide synthase; NO, nitric oxide.

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Reply

The comments of Cann *et al.* (1) bearing on the metabolic basis for the antineoplastic activity of L-canavanine are reasonable and worthy of thoughtful response. These authors create the impression that the incorporation of canavanine into proteins is questionable. In addition to the evidence presented in our paper, we add that over the years, we have demonstrated that L-[guanidinooxy-^{14}C]canavanine is incorporated into the *de novo*-synthesized proteins of every canavanine-sensitive organism that has ever been studied (2). In marked contrast, the neotropic bruchid beetle, *Caryedes brasiliensis*, which develops within canavanine-laden seeds, exhibits a remarkable discriminatory capacity to distinguish between arginine and canavanine (3), and scrupulously avoids canavanyl protein production (4). The destructive tobacco budworm, *Heliothis virescens*, which does not consume canavanine-containing plants, but nevertheless possesses an extraordinary resistance to canavanine, also fails to produce significant canavanyl proteins (5). The body of evidence accumulated over years of careful study leaves little doubt of the importance of aberrant, canavanyl protein formation in the expression of canavanine's antimetabolic properties (6–8). Finally, we have demonstrated that canavanine-treated rats incorporate canavanine into their proteins, and that the radiolabeling of the proteins of the pancreas is far greater than the tissues of all other studied organs (9).

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Cann *et al.* (1) afford three alternate bases for canavanine's toxicity. The first is its ability to function as a competitor of arginine for nitric oxide synthase. Our experience is that canavanine is not an effective inhibitor of this enzyme system. Within the past year, this question was reinvestigated independently by workers at the NIH at our request, they reached the same conclusion. Second, over the years, we have studied the ability of canavanine to function as a substrate for a wide variety of arginases. In the rat, highly purified arginase exhibits a V_{max} that is nearly 32 times greater with arginine than with canavanine. Bovine arginine-dependent arginase is less active than the rat, but the canavanine-dependent activity cannot even be detected (10). The jack bean, *Canavalia ensiformis*, a canavanine-storing legume, utilizes a common arginase to hydrolyze both arginine and canavanine (11). The apparent K_m for arginine of 7–8 mM increases to 38 mM for canavanine. In a canavanine-free plant such as soybean, *Glycine max*, the K_m for canavanine is so high that the formation of canaline, the product of canavanine-dependent hydrolysis, remains linear even when the canavanine concentration reaches a staggering 890 mM (11).

Canavanine is not an effective inhibitor of polyamine metabolism but L-canaline is. The latter nonprotein amino acid reacts aggressively with the pyridoxal phosphate moiety of B_6-dependent enzymes to form a stable covalently bound oxime that inactivates the enzyme (12). As little as 10^{-7} M canaline reduces aminotransferase activity by 75% after a 10 min incubation. We recognize that some of the toxicity, as well as the anticancer potential of canavanine, may be derived from canaline. This point is under active study at this time.

We accept the argument that other factors undoubtedly contribute to the antimetabolic properties of canavanine, but the preponderance of evidence is consistent with the importance we placed on the formation of structurally aberrant, canavanine-containing macromolecules.

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