

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 ARG¹ 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.

Stephen A. Cann
Johannes P. van Netten
Andrew S. Ross
Immunoassay and Special Development Laboratory
Greater Victoria Hospital Society
Royal Jubilee Hospital
Victoria, British Columbia, Canada

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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-¹⁴C]canavanine is incorporated into the *de novo*-synthesized proteins of every canavanine-sensitive organism that has ever been studied (2). In marked contrast, the neotropical 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 canavanil 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 canavanil proteins (5). The body of evidence accumulated over years of careful study leaves little doubt of the importance of aberrant, canavanil 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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Stephen A. Cann, Johannes P. van Netten and Andrew S. Ross

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