Advances in Brief

Inhibition of the Growth of Human Pancreatic Cancer Cells by the Arginine Antimetabolite L-Canavanine[1]

## Diane Shomin Swaffar,[2] Choo Yaw Ang, Pankaj B. Desai, and Gerald A. Rosenthal

Divisions of Pharmacology and Toxicology [D. S. S., C. Y. A.] and Pharmaceutics and Medicinal Chemistry [P. B. D.], School of Pharmacy, Northeast Louisiana University, Monroe, Louisiana 71209-0470, and T. H. Morgan School of Biological Sciences, University of Kentucky, Lexington, Kentucky 40506 [G. A. R.]

Abstract

L-Canavanine (CAV), the L-2-amino-4-guanidinooxy structural analogue of L-arginine (ARG), is a potent ARG antagonist which occurs in the jack bean, *Canavalia ensiformis*. This ARG antimetabolite is active against L1210 murine leukemia and a solid colonic tumor in the rat. Our initial studies using a microtiter assay show that CAV exhibits a 50% inhibitory concentration of approximately 2 mM against the human pancreatic adenocarcinoma cell line, MIA PaCa-2, when these cells are grown in Dulbecco's modified Eagle's medium containing 0.4 mM ARG. When the ARG concentration is reduced to 0.4 μM, the 50% inhibitory concentration for CAV falls precipitously to 0.01 mM. The pronounced increase in the ability of CAV to inhibit MIA PaCa-2 cell growth at the lower ARG concentration may result from enhanced CAV competition with ARG for incorporation into newly synthesized cellular proteins. At 0.4 μM ARG, 30 mM CAV almost completely inhibits cell growth by 6 h. In contrast, with 0.4 mM ARG, complete inhibition does not occur until after 48 h. A dramatic reversal of growth inhibition caused by a very high concentration of CAV was observed when cells treated with CAV were replenished with a high concentration of ARG. Our results suggest that CAV has real potential as a lead compound for the development of analogues with enhanced activity against human pancreatic cancer.

Introduction

CAV[3] [L-2-amino-4-(guanidinooxy)butyric acid] is a structural analogue of ARG originally isolated from the jack bean, *Canavalia ensiformis* (1). It differs structurally from ARG in that oxygen replaces the terminal methylene group of ARG.

L-canavanine
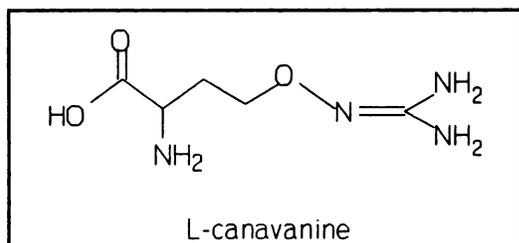
CAV is synthesized by many leguminous plants and plays a seminal role in higher plant chemical defense against insects (2). CAV, a

substrate for arginyl-tRNA synthetase (3), is incorporated readily into proteins in place of ARG (4). In insects, Rosenthal *et al.* (5–7) and coworkers have shown that this incorporation produces structurally aberrant "canavanyl" proteins which exhibit altered protein conformation (5) and impaired function (6, 7). This nonprotein amino acid is much less basic than ARG; the guanidinooxy group of CAV has a pK_a of 7.05 (8), while that of ARG is 12.48 (9). Incorporation of CAV into newly synthesized proteins can decrease residue basicity and alter residue interactions, thereby disrupting tertiary and/or quaternary protein structure.

Numerous studies on the effects of CAV have been conducted in insects and prokaryotes (reviewed in Refs. 2 and 6) with fewer reports of effects on mammalian cells. Kruse and McCoy (10) demonstrated that CAV affects Walker carcinosarcoma 256 cells; it was shown later to have antitumor activity against murine L1210 leukemia (11). CAV was also found to sensitize human colon tumor cells to the lethal effects of γ-irradiation (12). These investigators reported that the lethal effects of CAV occur preferentially in rapidly dividing cells. In other studies with monkey kidney cells whose transformation was temperature dependent, CAV inhibited DNA synthesis to a greater extent in transformed *versus* normal (untransformed) cells (13, 14). Thomas *et al.* (15) have reported that CAV inhibited tumor growth in a solid rat colonic tumor model. *In vivo* studies with radiolabeled CAV in rats by Thomas and Rosenthal (16) have shown that CAV sequesters in pancreatic proteins. Most recently, Mattei *et al.* (17) found that CAV decreased survival of human melanoma cells and rendered these cells more sensitive to heat.

Based on the cited investigations, we sought to determine if CAV possesses cytotoxicity for human pancreatic cancer cell lines. For our initial studies on cytotoxic selectivity, we selected the human pancreatic adenocarcinoma cell line, MIA PaCa-2, originally described by Yunis *et al.* (18). We have assessed the sensitivity of MIA PaCa-2 pancreatic cancer cells to CAV and evaluated the palliative ability of ARG toward CAV toxicity. This is the first report of the activity of CAV in a human pancreatic cancer cell line and the ameliorating effect of ARG on CAV cytotoxicity.

Materials and Methods

Drugs and Chemical Reagents. CAV, isolated from seeds of the jack bean, *Canavalia ensiformis*, was purified by repetitive recrystallization (19) and dissolved in sterile PBS. MTT reagent was purchased from Sigma Chemical Co. (St. Louis, MO). Media reagents were purchased from GIBCO/BRL (Grand Island, NY).

Cell Culture. Our initial studies with MIA PaCa-2 cells were performed on cells grown in DMEM containing 4.5 g/liter glucose. This medium was supplemented with a penicillin/streptomycin solution (100 units/ml and 100 μg/ml, respectively), 10% calf serum [consisting of 10 parts fetal bovine serum and 90 parts newborn calf serum (GIBCO)], 0.25 μg/ml Fungizone, and 1% (w/v) L-glutamine. The cells were maintained at 37°C in a humidified 5% CO_2 atmosphere and subcultured every 4 to 5 days.

Since MIA PaCa-2 cells could not be maintained in an ARG-free medium, using GIBCO's formulation for DMEM, media which have a reduced ARG

content were prepared. For the initial experiments, this ARM contained 10-fold (0.04 mM), 100-fold (0.004 mM), or 1000-fold less (0.4 μM) ARG than that contained in the commercial DMEM (0.4 mM). For later experiments, ARG concentrations that fell between the above concentrations were used in the ARMs. Cells were maintained routinely in commercial DMEM and then exposed to ARM as described during assays.

MTT Assay for Cytotoxicity. The protocol used for the MTT assay was similar to those reported previously by Swaffar *et al.* (20, 21) with several modifications. MIA PaCa-2 cells grown in DMEM were trypsinized with 0.05% (w/v) trypsin and 0.53 mM EDTA prior to centrifugation at 1000 rpm for 5 min. After cells were washed twice with ARM, the cell pellet was resuspended in fresh ARM. Cells were counted with a hemacytometer, and viability was determined by trypan blue exclusion (only cells having a viability >95% were used for assays). The cell suspension was adjusted to a concentration of 3×10^5 cells/ml by the addition of the appropriate amount of ARM. Using a multichannel pipettor, 100 μl of this cell suspension were plated into a 96-well microtiter plate. Wells containing only medium served as blanks. Microtiter plates were then incubated in a humidified 5% CO_2 atmosphere at 37°C for 4 h to allow the cells to adhere. Eight replicate samples, consisting of two sets of quadruplicate wells, were used for each CAV concentration (11 μl/well); control wells received PBS. Plates were incubated at 37°C in a humidified 5% CO_2 atmosphere. After 68 h, the contents of the wells were aspirated and replaced with fresh, unsupplemented McCoy's medium (ARM and DMEM interfered with the MTT assay). A 5-mg/ml (11-μl) stock solution of MTT in PBS was added to all wells. Plates were incubated for 4 h, and 100 μl of 0.04 N HCl in isopropyl alcohol was added and thoroughly mixed to solubilize the formazan produced by viable cells. Well absorbance was measured at 540 nm with a Bio-Rad MP450 plate reader. The mean absorbance of quadruplicate drug-treated wells was compared to that of control wells and expressed as a percentage of control absorbance ± SEM. For each ARG concentration, at least three independent experiments were performed.

Timed-Exposure Studies. In order to determine the time course of CAV-mediated growth inhibition at varying ARG concentrations, MIA PaCa-2 cells were exposed to CAV in ARM or DMEM and were plated as above. After an incubation period of at least 4 h, the upper four rows of the plate received 11 μl of CAV solution, and quadruplicate wells on the lower four rows of the plate received 11 μl PBS. Plates were incubated for the specified period of time. At each time point, CAV- or PBS-containing medium was aspirated from the appropriate wells and replaced with fresh medium. Plates were then incubated for the remaining period of the assay, the medium was aspirated and replaced with unsupplemented McCoy's medium, MTT added, and absorbances were measured as above. The data were processed as described above.

In order to determine if cell growth inhibition due to CAV was reversed by ARG, cells were exposed to CAV in a medium containing 0.4 μM ARG (1000-fold less ARG than that of DMEM). MIA PaCa-2 cells in ARM containing 0.4 μM ARG were plated as above, treated with either CAV or PBS as described above, and incubated for the indicated time periods. At each time point, CAV- or PBS-containing medium was aspirated from the appropriate wells and replaced with either fresh DMEM or ARM with 0.4 μM ARG. Plates were incubated for the remaining period of the assay and processed as above.

Results

The sensitivity of MIA PaCa-2 cells to CAV after a 72-h exposure in DMEM is depicted in Fig. 1. In this medium, CAV had only moderate cytotoxicity against MIA PaCa-2 cells, as demonstrated by an IC_{50} of around 2.5 mM. CAV at 0.1 mM did not inhibit the growth of any of the cells, but nearly 80% of the cells were killed by approximately 3 mM CAV; complete inhibition of cell growth was seen at 30 mM CAV.

Fig. 2 illustrates the results of varying the concentration of ARG on MIA PaCa-2 cell growth. As the ARG concentration of the media decreased, CAV toxicity was amplified. CAV toxicity increased about 10-fold from a 10-fold reduction in ARG concentration (0.04 mM), but CAV toxicity did not increase exponentially when the ARG concentration was lower than 0.004 mM. CAV was nearly 200-fold more toxic when assays were performed at the lowest ARG concentration (0.4 μM) evaluated in our study. In this instance, the IC_{50} level was
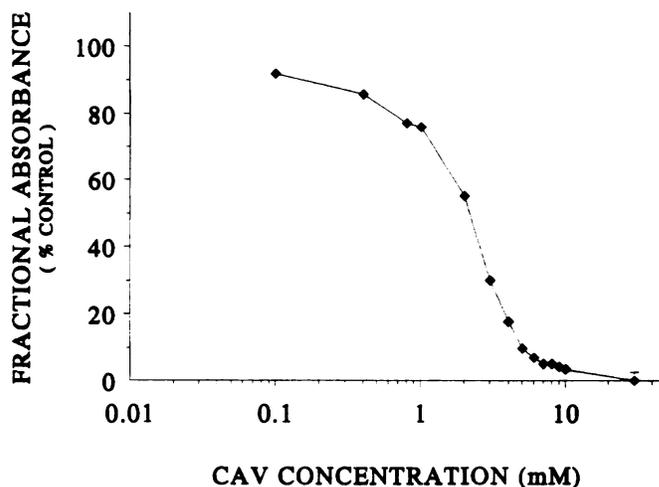

Fig. 1. Sensitivity of MIA PaCa-2 cells to CAV after 72-h exposure in DMEM (0.4 mM ARG). Cell survival was determined by the MTT assay as described in "Materials and Methods." *Points*, the means of 10 independent experiments; *bars*, SE. The SE bar was omitted if it fell within the region occupied by the data point.

approximately 15 μM. At an ARG concentration of 0.8 μM, 0.004 mM, or 0.008 mM, the CAV IC_{50} was only slightly higher, but when the ARG concentration reached 0.04 mM, the IC_{50} rose to 0.35 mM. At 0.4 mM ARG, the CAV IC_{50} reached about 2.5 mM.

MIA PaCa-2 cells were exposed to a concentration of CAV (30 mM) that was shown in earlier experiments to inhibit the growth of 100% of the cells after 72 h in DMEM. At each of the indicated time periods of Fig. 3, CAV-containing medium was removed and replaced with fresh medium; the cells were further incubated for a total exposure period of 72 h. When cells were exposed to 30 mM CAV (in DMEM), growth inhibition exceeded 50% after 18 h. In contrast, at 0.04 mM ARG, 50% of the cells were killed after only 1 h, and by 24 h, over 80% growth inhibition was seen. When the ARG concentration was lowered to 0.4 μM, cell growth inhibition attained 80% after 1 h. Nearly all cells were killed by 18 h when CAV exposure occurred with the lowest ARG concentration evaluated.

The dramatic reversal of CAV toxicity by ARG is depicted in Fig. 4. This figure depicts the results of a timed-exposure study conducted with cells exposed to 30 mM CAV in 0.4 μM ARG-containing medium. The CAV-containing medium was removed at the indicated times and replaced with either ARM (0.4 μM ARG) or DMEM. Cells were then incubated for a total exposure period of 72 h. These results clearly showed that, at earlier time points, CAV toxicity was reversed by ARG. Interestingly, over 80% of the cell growth was inhibited when cells were exposed to the low ARG media for 1 h and then replaced with this same media. However, when cells were exposed to the low ARG media for 1 h and then replaced with DMEM containing 0.4 mM ARG, no cell growth inhibition was seen. This dramatic reversal continued up to about 12 h, and by 18 h, there was still partial reversal of cell growth inhibition by ARG. After 24 h of exposure of cells to CAV, ARG could no longer reverse CAV-mediated cell growth inhibition. In contrast, nearly complete reversal of growth inhibition was still seen when cells were exposed to CAV in the presence of 0.4 μM ARG for 6 h and then replaced with DMEM.

Discussion

CAV inhibits the growth of MIA PaCa-2 cells in a dose-dependent manner, but the degree of growth inhibition is dependent on the ARG concentration of the cell growth medium. Concentrations of ARG from 0.04 mM to 0.4 mM assuage CAV toxicity. The high IC_{50} of CAV
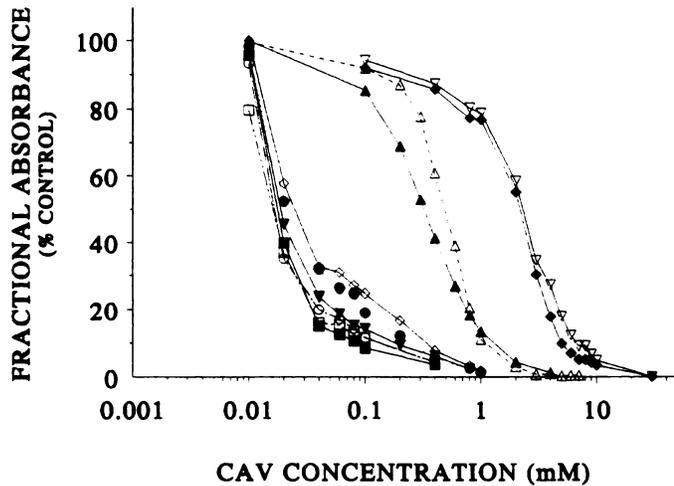
6046

Fig. 2. Sensitivity of MIA PaCa-2 cells exposed to CAV for 72 h in ARM containing varying concentrations of ARG: ▽, 0.4 mM; △, 0.08 mM; ▲, 0.04 mM; ◇, 0.03 mM; ●, 0.02 mM; ▼, 0.008 mM; ○, 0.004 mM; ■, 0.08 μm; and □, 0.04 μM. ◆, DMEM. Cell survival was determined as in Fig. 1. *Points*, the means of results from 3 to 10 independent experiments. SE bars are omitted for clarity. (SEMs ranged from 0 to 6.9%).

destroy them; however, our results suggest otherwise. Our hypothesis is that, if proteins that have incorporated CAV are rapidly degraded, at a continuing low ARG concentration, insufficient ARG is present to preferentially be taken up into protein, and this is a toxic insult to the cells. Pazlarova *et al.* (27) have recently reported that, in *Saccharomyces cerevisiae,* CAV-containing proteins were degraded more slowly when glucose was in the medium as compared to ethanol (as a carbon source) in the medium when ARG was added after a 2-h ARG-free incubation. This interesting observation warrants further investigation of protein degradation in the MIA PaCa-2 cells treated with and without CAV in the presence of varying ARG concentrations.

The question of why CAV-mediated inhibition of MIA PaCa-2 cells is reversible with ARG up to 12 h posttreatment but then becomes irreversible is intriguing and worthy of detailed investiga-

seen in DMEM was undoubtedly due to direct competition with ARG in the cell growth medium. When ARG is present in an adequate concentration, it probably competes effectively with CAV for uptake into cellular proteins, and this attenuates the toxic effects of CAV. However, the issue of whether the rate of CAV incorporation into cellular protein has actually been altered by the ARG concentrations used awaits CAV uptake experiments. Our results suggest that more CAV was probably incorporated into protein when ARG concentrations were low. Under these conditions, it is likely that a greater extent of CAV incorporation would result in a greater degree of cell growth inhibition.

Timed-exposure studies were performed to determine when CAV begins to exhibit growth inhibitory effects, both with high and trace amounts of ARG. In the presence of 0.4 mM ARG, a concentration of CAV, known to inhibit the growth of 100% of the cells by 72 h, inhibits the growth of 50% of the cells around 18 h. Up to 12–18 h, CAV toxicity appears to be readily reversed by ARG. Even the effects of a highly toxic dose such as 30 mM CAV can be nearly completely reversed by 0.4 mM ARG. For example, when cells exposed to CAV for 6 h in the low ARG media were then washed free of CAV and replenished with a high amount of ARG (as in DMEM), the ARG apparently was able to almost completely reverse the cell growth inhibition (compared to that seen when cells were exposed to CAV and replenished with a lower amount of ARG).

We found that replenishment of MIA PaCa-2 cells with normal amounts of ARG (as in DMEM) can reverse completely cell growth inhibition at early time points and that this becomes irreversible after approximately 18 h. Finally, our study provides the first evidence of the activity of CAV in a human pancreatic carcinoma and of ARG reversibility of growth inhibition in a tumor cell line. The results of our study of CAV and MIA PaCa-2 cells suggest that CAV and ARG compete directly for activation and aminoacylation by arginyl-tRNA synthetase. We plan to evaluate this point by providing these cells with L-[*guanidinooxy*-^{14}C]CAV and monitoring its uptake into newly synthesized proteins in the presence of varying amounts of ARG.

These results also pose the question of what influence ARG concentrations may have on the rate of turnover of proteins that have incorporated CAV. Several investigators have reported that some CAV-containing proteins degrade faster than normal proteins (22–26). Cells then may have the ability to recognize aberrant proteins and
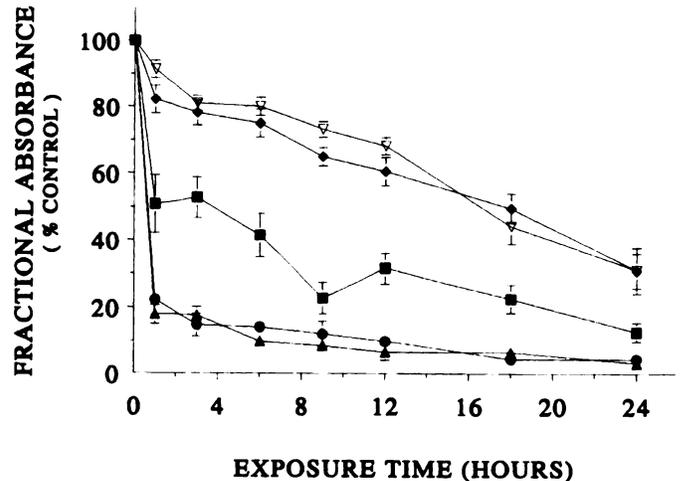

Fig. 3. The time course of growth inhibition of MIA PaCa-2 cells by CAV in ARM containing varying concentrations of ARG. CAV-containing medium was removed after the indicated times and replaced with fresh medium. Cells were further incubated for a total exposure period of 72 h, and cell survival was determined. ◆, DMEM; ▽, ARM (0.4 mM); ■, ARM (0.04 mM; ●, ARM (0.004 mM); and ▲, ARM (0.4 μM). *Points*, the means of results obtained from at least three independent experiments; *bars*, SE.
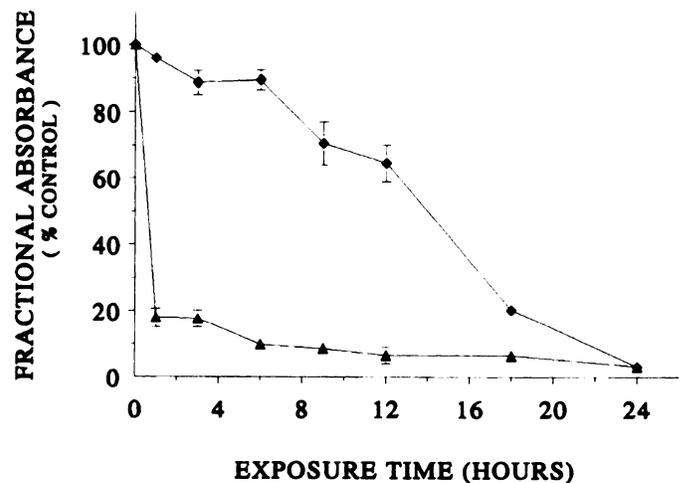

Fig. 4. Reversal of cell growth inhibition by ARG. MIA PaCa-2 cells were exposed to 30 mM CAV in ARM containing 0.4 μM ARG for the indicated time periods. CAV-containing medium was removed and replaced with either fresh ARM with 0.4 μM ARG or with fresh DMEM (0.4 mM ARG). Cells were incubated for a total exposure period of 72 h. ◆, replaced with DMEM; ▲, replaced with 0.4 μM ARG media. *Points*, the means of results obtained from at least three independent experiments; *bars*, SE. This clearly showed that, at earlier time points, CAV toxicity was reversible by ARG. After 24 h, it became irreversible.

tion. These cells would be expected to preferentially degrade their CAV-containing proteins and thereby remove such structurally aberrant proteins. Perhaps, there is a threshold for effective error correction, and the production of excess aberrant protein countervails cellular ability to remove these defective macromolecules. Alternatively, CAV may disrupt nucleic acid metabolism, and a point may be reached where ARG-driven cellular recovery is no longer possible. We plan to examine radiolabeled thymidine and uridine uptake by CAV-treated and control cells and to determine the turnover and degradation of [^{3}H]ARG- and [^{14}C]CAV-containing proteins.

CAV has been reported to induce the synthesis of heat shock proteins (28, 29). It is not known what effect ARG concentrations in the medium have on levels of heat shock proteins, but based on these reports, it would be reasonable to expect that lower concentrations of ARG in the medium would increase the synthesis of heat shock proteins, i.e., at the lower ARG concentrations, more hsps would be produced, and this may enable cells to survive at higher temperatures. However, other investigators have reported that CAV renders melanoma cells more sensitive to heat (17). Also, Laszlo and Li (30) have recently reported that, in Chinese hamster fibroblasts, CAV induces synthesis of hsps, but these hsps are nonfunctional and sensitize the cells to heat. It is not known what effect heat has on our MIA PaCa-2 cells exposed to CAV, since all our studies were conducted at 37°C.

Overall, our preliminary report of the activity of CAV against a human pancreatic cancer cell line suggests that CAV may be incorporated in place of ARG in ARG-rich pancreatic proteins and contribute to the demise of these cells. We have also clearly demonstrated that CAV toxicity to MIA PaCa-2 cells is reversible by ARG but after 18 h becomes irreversible. Studies to address these interesting observations are in progress.

References

1. Kitagawa, M., and Tomiyama, T. A new amino compound in the jack bean and a corresponding new ferment. J. Biochem. (Tokyo), *11:* 265–271, 1929.
2. Rosenthal, G. A. The biological effects and mode of action of L-canavanine, a structural analogue of L-arginine. Q. Rev. Biol., *52:* 155–178, 1977.
3. Allende, C. C., and Allende, J. E. Purification and substrate specificity of arginyl tRNA synthetase from rat liver. J. Biol. Chem., *239:* 1102–1112, 1964.
4. Rosenthal, G. A. Nonprotein amino acids in the life processes of higher plants. *In:* B. K. Singh, H. E. Flores, and J. C. Shannon (eds.), Biosynthesis and Molecular Regulation of Amino Acids in Plants, pp. 249–261. Rockville, MD: American Society of Plant Physiologists, 1992.
5. Rosenthal, G. A., Reichhart, J-M., and Hoffmann, J. A. L-Canavanine incorporation into vitellogenin and macromolecular conformation. J. Biol. Chem., *264:* 13693–13696, 1989.
6. Rosenthal, G. A., Lamber, J., and Hoffmann, D. Canavanine incorporation into the antibacterial proteins of the fly, *Phormia terranovae* (Diptera), and its effect on biological activity. J. Biol. Chem., *264:* 9768–9771, 1989.
7. Rosenthal, G. A., and Dahlman, D. L. Studies of L-canavanine incorporation into insectan lysozyme. J. Biol. Chem., *266:* 15684–15687, 1991.
8. Boyar, A. and Marsh, R. E. L-Canavanine, a paradigm for the structures of substituted guanidines. J. Am. Chem. Soc., *104:* 1995–1998, 1982.
9. Greenstein, J. P., and Winitz, M. Chemistry of the Amino Acids, Vol. 3, pp. 2622–2628. New York: John Wiley & Sons, Inc., 1961.
10. Kruse, P. R., and McCoy, T. A. The competitive effect of canavanine on utilization of arginine in growth of Walker carcinosarcoma 256 cells *in vitro.* Cancer Res., *18:* 279–282, 1958.
11. Green, M. H., Brooks, T. L., Mendelsohn, J., and Howell, S. B. Anti-tumor activity of L-canavanine against L1210 murine leukemia. Cancer Res., *40:* 535–537, 1980.
12. Green, M. H., and Ward, J. F. Enhancement of human tumor cell killing by L-canavanine in combination with τ-radiation. Cancer Res., *43:* 4180–4182, 1983.
13. Naha, P. M., Silcock, J. M., and Fellows, L. Reappraisal of L-canavanine as an anti-tumor agent. Cell Biol. Int. Rep., *4:* 155–166, 1980.
14. Naha, P. M. An experimental model for selective inhibition of proliferating cells by chemotherapeutic agents. Cell Biol. Int. Rep., *4:* 829–839, 1980.
15. Thomas, D. A., Rosenthal, G. A., Gold, D. V., and Dickey, K. Growth inhibition of a rat colon tumor by L-canavanine. Cancer Res., *46:* 2898–2903, 1986.
16. Thomas, D. A., and Rosenthal, G. A. Toxicity and pharmacokinetics of the nonprotein amino acid L-canavanine in the rat. Toxicol. Appl. Pharmacol., *91:* 395–405, 1987.
17. Mattei, E., Damasi, D., Mileo, A. M., Delpino, A., and Ferrini, U. Stress response, survival and enhancement of heat sensitivity in a human melanoma cell line treated with L-canavanine. Anticancer Res., *12:* 757–762, 1992.
18. Yunis, A. A., Arimura, G. K., and Russin, D. J. Human pancreatic carcinoma (MIA PaCa-2) in continuous culture: sensitivity to asparaginase. Int. J. Cancer, *19:* 128–135, 1977.
19. Rosenthal, G. A. Preparation and colorimetric analysis of L-canavanine. Anal. Biochem., *77:* 147–151, 1977.
20. Swaffar, D. S., Horstman, M. G., Jaw, J., Thrall, B. D., Meadows, G. C., Harker, W. G., and Yost, G. S. Methylazoxyprocarbazine, the active metabolite responsible for the anticancer activity of procarbazine against L1210 leukemia. Cancer Res., *49:* 2442–2447, 1989.
21. Swaffar, D. S., Ireland, C. M., and Barrows, L. R. A rapid mechanism-based screen to detect potential anti-cancer agents. Anti-Cancer Drugs, *5:* 15–23, 1994.
22. Prouty, W. F., Karnovsky, M. J., and Goldberg, A. L. Degradation of abnormal proteins in *Escherichia coli:* formation of protein inclusions in cells exposed to amino acid analogs. J. Biol. Chem., *250:* 1112–1122, 1975.
23. Prouty, W. F. Degradation of abnormal proteins in HeLa cells. J. Cell Physiol., *88:* 371–382, 1976.
24. Fong, D., and Poole, B. The effect of canavanine on protein synthesis and protein degradation in IMR-90 fibroblasts. Biochim. Biophys. Acta, *696:* 193–200, 1982.
25. Halban, P. A., Amherdt, M., Orci, L., and Renold, A. E. Proinsulin modified by analogues of arginine and lysine is degraded rapidly in pancreatic B-cells. Biochem. J., *219:* 91–97, 1984.
26. Rosenthal, G. A., and Dahlman, D. L. L-Canavanine and protein synthesis in the tobacco hornworm *Manduca sexta.* Proc. Natl. Acad. Sci. USA, *83:* 14–18, 1986.
27. Pazlarova, J., Dvorakova, M., and Chaloupka, J. Turnover of canavanine-containing proteins in *Saccharomyces cerevisiae.* Folia Microbiol. Praha, *38:* 225–228, 1993.
28. DiDomenico, B. J., Bugaisky, G. E., and Lindquist, S. The heat shock response is self regulated at both the transcriptional and post transcriptional levels. Cell, *31:* 593–603, 1982.
29. Amaral, M. D., Galego, L., and Rodrigues, P. C. Heat-shock-induced protein synthesis is responsible for the switch-off of hsp70 transcription in Tetrahymena. Biochim. Biophys. Acta, *1174:* 133–142, 1993.
30. Laszlo, A., and Li, G. C. Effect of amino acid analogs on the development of thermotolerance and on thermotolerant cells. J. Cell. Physiol., *154:* 419–432, 1993.

Inhibition of the Growth of Human Pancreatic Cancer Cells by the Arginine Antimetabolite l-Canavanine

Diane Shomin Swaffar, Choo Yaw Ang, Pankaj B. Desai, et al.

Updated version	Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/54/23/6045

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/54/23/6045. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.