

# UC Irvine

## UC Irvine Previously Published Works

### Title

Polyamine biosynthesis inhibitors combined with systemic hyperthermia in cancer therapy

### Permalink

<https://escholarship.org/uc/item/1wt2d2kq>

### Journal

International Journal of Radiation Oncology • Biology • Physics, 19(1)

### ISSN

0360-3016

### Authors

Harari, Paul M  
Fuller, David JM  
Carper, Stephen W  
[et al.](#)

### Publication Date

1990-07-01

### DOI

10.1016/0360-3016(90)90139-b

### Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

● *ASTRO 1989 Resident Essay Award*

## POLYAMINE BIOSYNTHESIS INHIBITORS COMBINED WITH SYSTEMIC HYPERTHERMIA IN CANCER THERAPY

PAUL M. HARARI, M.D., DAVID J. M. FULLER, PH.D., STEPHEN W. CARPER, PH.D.,  
MARILYN K. CROGHAN, M.D., FRANK L. MEYSKENS JR., M.D.,  
DAVID S. SHIMM, M.D. AND EUGENE W. GERNER, PH.D.

The University of Arizona Cancer Center, Department of Radiation Oncology and  
Department of Medicine, Section of Medical Oncology, Tucson, AZ 85724

A Phase I clinical trial has been initiated at the University of Arizona Cancer Center which combines escalating oral doses of the polyamine biosynthesis inhibitor  $\alpha$ -difluoromethylornithine (DFMO), with systemic hyperthermia ( $\sim 41.5^\circ\text{C}$ ) in the treatment of metastatic melanoma. The rationale for the combination of hyperthermia and polyamine biosynthesis inhibitors in the treatment of human cancers includes studies which show that depletion of endogenous polyamines, as a result of treatment with DFMO, sensitizes both rodent and human tumor cells to the cytotoxic effects of hyperthermia. Heat shock induces the first enzyme in polyamine catabolism, spermidine/spermine  $\text{N}^1$ -acetyltransferase ( $\text{N}^1$ -SAT). The consequently acetylated forms of spermidine and spermine are then constitutively oxidized by the enzyme polyamine oxidase (PAO). Both CHO and human A549 lung cancer cells exhibit heat-inducible polyamine acetylation, display potent heat sensitization after polyamine depletion, and ultimately reveal prolonged expression of thermotolerance. Conversely, HeLa cells do not demonstrate heat-inducible polyamine catabolism, are not sensitized to heat with DFMO, and display more rapid kinetics of thermotolerance decay. These laboratory studies suggest that enhancement of the cytotoxic action of hyperthermia by DFMO occurs as a consequence of the inhibition of polyamine catabolism, a heat-inducible process that affords some form of protection to cells undergoing heat stress. Human melanoma cultures demonstrate heat-inducible polyamine catabolism and are sensitized to hyperthermic cytotoxicity by DFMO. To date, 24 systemic hyperthermia treatments have been delivered to nine patients with metastatic melanoma in conjunction with oral DFMO under this Phase I clinical trial.

Systemic hyperthermia, Polyamines, DFMO, Melanoma.

### INTRODUCTION

The naturally occurring polyamines play an essential role in the growth, proliferation and differentiation of mammalian cells (16, 24). A simplified schematic of polyamine metabolism is depicted in Figure 1. Manipulation of polyamine metabolism through the use of enzyme inhibitors has been under investigation for its potential utility in anticancer therapies. DFMO, the irreversible inhibitor of ornithine decarboxylase (ODC), the first enzyme in polyamine biosynthesis, has been studied for its antiproliferative activity alone, and in combination with a variety of anticancer agents (1, 2, 4, 14, 16, 22). DFMO has further been implicated as an antimetastatic agent based on its

capacity to inhibit pulmonary metastases in mice (23). Recently, a specific, irreversible inhibitor of polyamine catabolism has been synthesized (polyamine oxidase inhibitor [PAOI], MDL-72.521), and thus the physiological significance of this pathway is under evaluation as well (3).

The clinical use of hyperthermia to treat human cancer has advanced markedly in recent years due to improvements in hyperthermia technology, and due to progress in our understanding of the mechanisms of synergy between heat and other anticancer agents. The relationship between heat sensitivity and polyamine metabolism has been a focus of our laboratory, with particular regard to

Reprint requests to: Eugene W. Gerner, Ph.D., Department of Radiation Oncology, University of Arizona Cancer Center, Tucson, AZ 85724.

*Acknowledgements*—Mr. Paul Ruggera of the Center for Devices and Radiological Health, FDA provided the CDRH Helix. T. Cetas, M. Gopal, D. Anhalt and D. Buechler prepared the device for clinical use. Special thanks to Dr. S. Salmon and his labo-

ratory for providing the human tumor cell clonogenic assay data from melanoma biopsies. Expert preparation of the manuscript was provided by Leslie Pike. Supported by grants from USPHS National Cancer Institute, CA-17343, CA-30052, CA-09213 and an award from the Arizona Disease Commission.

Accepted for publication 24 January 1990.

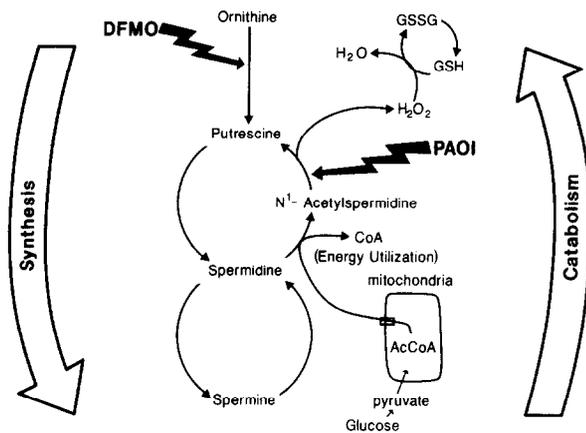


Fig. 1. Schematic of polyamine metabolism citing location of synthesis inhibition by  $\alpha$ -difluoromethylornithine (DFMO), and catabolism inhibition by polyamine oxidase inhibitor (PAOI).

methods of enhancing hyperthermic cytotoxicity for therapeutic gain. It is known that depletion of endogenous polyamine pools, via synthesis blockade with DFMO, renders cells more sensitive to subsequent heat stress (7, 8). Further, pathways of polyamine catabolism are affected by thermal stress and appear to influence ultimate cell viability responses under certain conditions (9).

The present study was conducted to further establish the rationale for combining polyamine biosynthesis inhibitors with hyperthermia in the treatment of human cancers. Experimental results suggest that the mechanism of enhanced cytotoxicity due to DFMO/hyperthermia combinations may involve the inhibition of polyamine catabolism, a finding which has important implications for new inhibitor development. Studies with several human tumor and rodent cell lines suggest that the ability of heat shock to induce polyamine acetylation may be a useful marker to predict whether polyamine synthesis inhibitors will potentiate hyperthermia-induced cell killing. Finally, a Phase I clinical trial combining DFMO and systemic hyperthermia is introduced for the treatment of patients with metastatic melanoma.

## METHODS AND MATERIALS

### Cell culture

All cell cultures were maintained as monolayer cultures in McCoy's 5A medium supplemented with 10% fetal bovine serum plus 1% penicillin-streptomycin solution.\* Cultures were maintained at 37°C in humidified incubators with 5% CO<sub>2</sub>:95% air. The following cell lines were studied with approximate doubling times indicated; CHO 16–18 hr, A549 18–20 hr, Hela 22–24 hr. All experiments were initiated with exponentially growing cultures.

### Hyperthermia treatments

All heat shock treatments were performed by sealing T-25 or T-75 flasks<sup>†</sup> and immersing them in a precision temperature-controlled circulating water bath ( $\pm 0.1^\circ\text{C}$ ) for treatment times indicated in text.

### Polyamine analysis

Separation of the natural polyamines and their monoacetyl derivatives was achieved by reverse-phase high-performance liquid chromatography (HPLC) using the method of Seiler and Knodgen (19). All representative HPLC experiments were replicated a minimum of three times.

### Glutathione assay

Glutathione levels were measured by the method of Tietze (25), 6 hr following removal from 43°C heat stress. Cells were harvested, washed twice and resuspended at a concentration of  $6 \times 10^6$  cells/ml. After deproteinization with 0.6% sulfosalicylic acid and centrifugation at 0°C, the supernatant was assayed for glutathione content by spectrophotometry at 412 nm after addition of dithionitrobenzoic acid in the presence of glutathione reductase and NADPH. Values of glutathione in untreated CHO cultures ranged from 1.9 - 2.6  $\mu\text{g}/10^6$  cells.

### Spermidine N<sup>1</sup>-acetyltransferase (N<sup>1</sup>-SAT) assay

Enzyme activity was measured 6 hr following heat stress by a method adapted from Libby (11) and Matsui *et al.* (12). Cells were resuspended in 50 mM Tris pH 7.5, 2.5 mM DTT and 0.1 mM EDTA at a concentration of  $4 \times 10^7$  cells/ml. Each assay contained 3 mM spermidine, 50 mM Tris pH 7.8 and 8  $\mu\text{M}$ -[1-<sup>14</sup>C] Acetyl CoA in a total volume of 100  $\mu\text{l}$ . Activity was measured by the incorporation of <sup>14</sup>C label into spermidine from <sup>14</sup>C-Acetyl CoA and expressed as units per 10<sup>6</sup> cells, where 1 unit represents production of 1 pmol of N<sup>1</sup>-acetylspermidine per minute.

### Survival determinations

After heat shock or chemical treatments, known numbers of single cells were plated into 60 mm petri dishes in 5 ml of growth medium and incubated for 7–10 days. Colonies were stained with crystal violet and counted manually. All experimental groups were plated in triplicate. Plating efficiency for untreated controls ranged from 80–95%. Mean survival values were determined and plotted  $\pm$  standard error of the mean.

### Systemic hyperthermia

The CDRH Helix used for systemic hyperthermia treatments and the methods of patient preparation for whole body heating are previously described (10, 20, 21).

\* All from Grand Island Biological Co., Grand Island, NY.

<sup>†</sup> Parafilm "M", American Can Co., Greenwich, CT.

### Drugs and drug treatments

The following chemicals were purchased: buthionine S,R sulfoximine (BSO), dithiobis-nitrobenzoic acid (DTNB) sulfosalicylic acid,<sup>‡</sup> [1-<sup>14</sup>C]-Acetyl-CoA,<sup>§</sup> DFMO and the polyamine oxidase inhibitor, MDL-72.521 were gifts.\*\*

All treatments with the PAO inhibitors were carried out at a concentration of 25  $\mu$ M added directly to culture medium 2–4 hr prior to subsequent heat or drug treatments. For colony formation experiments, the PAO inhibitor (25  $\mu$ M) was present in dishes throughout the colony forming period. Buthionine S,R sulfoximine (10 mM) was added to cultures 24 hr prior to heat shock, but was not present during the colony forming interval. DFMO treatments were generally carried out at 5 mM concentrations with 8 hr exposure times 36 hr prior to survival experiments, and 48 hr continuously prior to enzyme studies.

## RESULTS

### Heat shock-induced polyamine acetylation

The effects of heat shock on polyamine oxidation were studied using a recently synthesized inhibitor of PAO, MDL-72.521. This specific, irreversible inhibitor of PAO blocks the intracellular oxidation of N<sup>1</sup>-acetylspermidine and subsequent formation of putrescine, 3-acetamidopropanal and hydrogen peroxide (3). Cultures receiving a 43°C  $\times$  90 minute heat shock, and then incubated at 37°C, displayed elevated putrescine levels during the 6 hr interval immediately following hyperthermic stress (Fig. 2). This rise in putrescine was completely blocked when cultures were treated with the PAO inhibitor. Further, N<sup>1</sup>-acetylspermidine levels increased in heat shocked cultures from undetectable levels to 1.5–2.0 nmol/mg protein in the presence of the PAO inhibitor. Spermidine contents did not vary substantially during these treatments, while spermidine levels decreased slightly in heat shocked cultures treated with the PAO inhibitor reflecting the formation of N<sup>1</sup>-acetylspermidine. These results demonstrate that the transient increase in putrescine content in cultures following heat stress is due to acetylation and subsequent oxidation of spermidine.

### Polyamine oxidation affects glutathione pools

The oxidation of N<sup>1</sup>-acetylspermidine by PAO results in the formation of putrescine, 3-acetamidopropanal (a reactive aldehyde), and hydrogen peroxide. Both of these latter two species are potentially cytotoxic and their detoxification may involve the cellular non-protein sulfhydryl, glutathione. Known detoxification reactions involving glutathione include the reduction of hydrogen peroxide to H<sub>2</sub>O producing GSSG (the oxidized form of

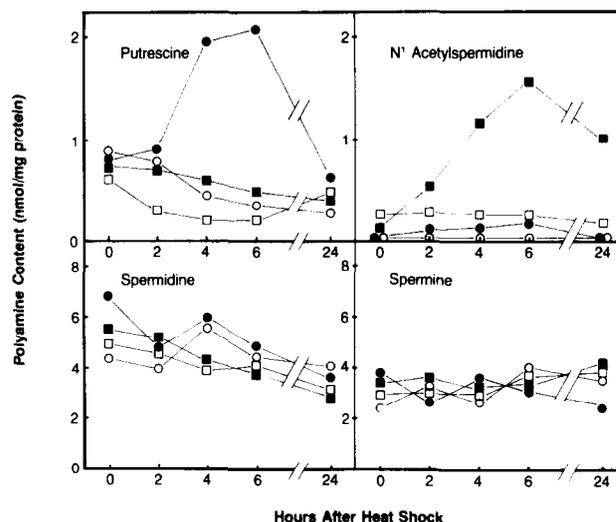


Fig. 2. Effects of heat shock on polyamine acetylation and oxidation. Log phase CHO cultures were heat shocked at 43°C for 90 min in the presence (■) or absence (●) of the PAO inhibitor (25  $\mu$ M). Control cultures at 37°C plus (□) or minus (○) the PAO inhibitor were also analyzed. Polyamine analysis was carried out via HPLC at times indicated after heat shock.

glutathione), and conjugation of glutathione to reactive moieties followed by their excretion from the cell (13). To determine whether glutathione was involved in any aspect of polyamine oxidation, levels of glutathione were measured after heat stress (Fig. 3).

Glutathione content was found to decrease in a manner dependent on both the time at euthermic temperature

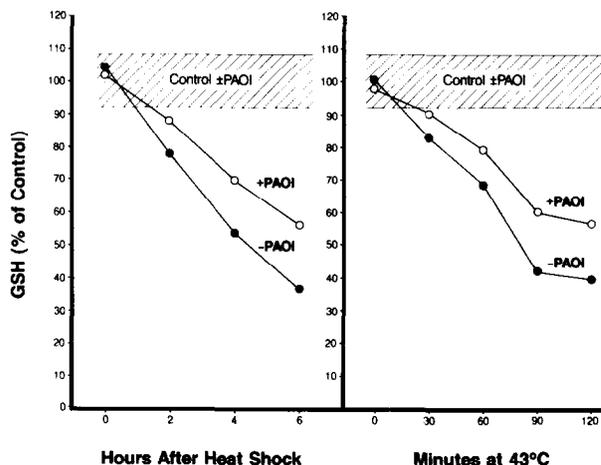


Fig. 3. Effects of heat shock on glutathione content. Log phase CHO cultures were heat shocked at 43°C for 90 min in the presence (○) or absence (●) of the PAO inhibitor and assayed for glutathione content at the indicated times after heat (left panel). Cultures that were heated at 43°C for various times were assayed for glutathione content at 6 hr after heat shock (right panel).

<sup>‡</sup> Sigma Chemical Co.

<sup>§</sup> New England Nuclear.

\*\* Merrell Dow Laboratories, Cincinnati, OH.

after heat shock, and the duration of the heat stress. Levels of glutathione decreased by 65% at 6 hr after cultures were returned to 37°C following exposure to 43°C for 90 minutes. Treatment of cultures with the PAO inhibitor partially inhibited this decrease in glutathione. The level of glutathione 6 hr after 43°C heat stress was also dependent on the duration of the hyperthermic treatment and decreases could be measured for exposures as short as 30 minutes. For all durations studied, the PAO inhibitor afforded a partial reduction in the observed decrease in glutathione levels.

#### *Polyamine oxidation and cell viability*

The PAO alone has no effect on the 43°C survival response of Chinese hamster cells as measured by colony formation (Fig. 4). However, a small protective effect on cell survival was observed in heat shocked cultures which were first depleted of cellular glutathione with the glutathione synthesis inhibitor, buthionine sulfoximine. This latter treatment, which is known to sensitize mammalian cells to the cytotoxic effects of heat (15), reduced glutathione levels by 70% from control values. This observation suggests that heat-induced endogenous polyamine oxidation can affect viability responses in glutathione depleted cultures, although polyamine oxidation is apparently not the sole participant in this hyperthermic toxicity.

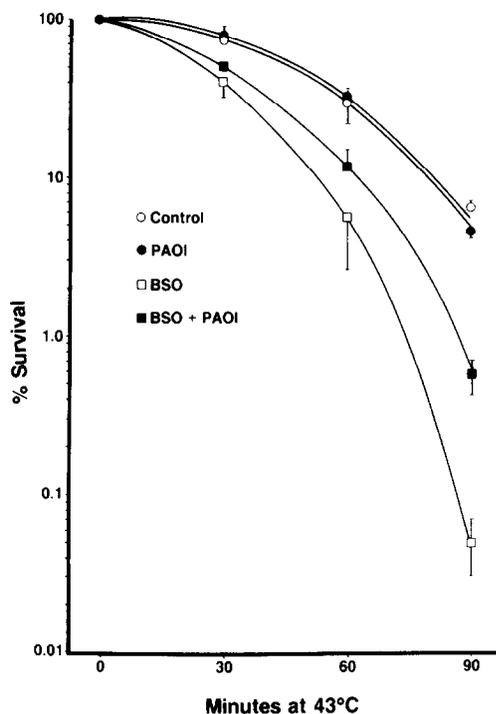


Fig. 4. Effects of PAO inhibition on survival of glutathione depleted cells. Log phase CHO cultures were plated for colony formation after heat shock at 43°C, preceded by various pretreatments. BSO (10 mM) was added 24 hr prior to heat shock and PAO inhibitor (25  $\mu$ M) was added 4 hr prior to heat shock and remained present throughout colony formation.

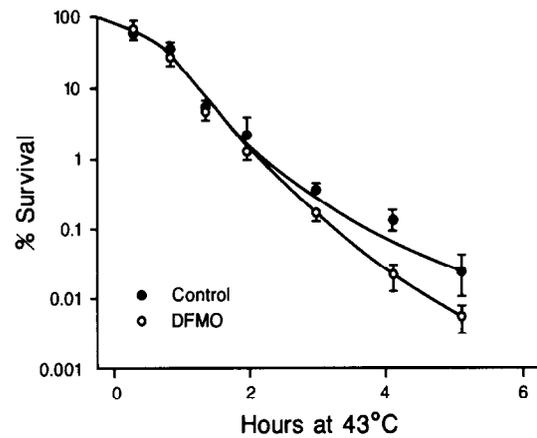


Fig. 5. HeLa cell colony survival at 43°C in control cultures (●), and in cultures treated with 5 mM DFMO for 8 hr (○). DFMO was then removed and cultures were incubated in fresh medium without drug for 36 hr prior to heat stress.

#### *Polyamine acetylation and DFMO-induced heat sensitization*

Figure 5 describes the 43°C survival response of HeLa cells (human cervix cancer) after polyamine depletion with DFMO. No appreciable difference in survival is observed comparing polyamine depleted cultures with controls. This is in distinct contrast to the results in CHO cultures (rodent line), where a 2 log sensitization to heat by DFMO is observed under identical conditions (7, 8). These findings have led us to further investigate the cell line specificity of heat shock and polyamine responses, with particular regard to the inducibility of polyamine acetylation by heat, and the sensitization to heat stress by polyamine depletion.

Table 1 outlines the correlation of heat-inducibility of polyamine acetylation ( $N^1$ -SAT induction) and heat sensitization by DFMO in one rodent (CHO) and two human (A549 and HeLa) cell lines. The CHO and A549 cell lines demonstrate heat-inducible polyamine acetylation (7–10 fold increase in  $N^1$ -SAT activity after heat stress), and this induction can be inhibited by pretreatment with DFMO. These cell lines also display significant heat sensitization (1–2 logs) after polyamine depletion with DFMO. The HeLa cells show no measurable induction of polyamine acetylation after heat stress, and exhibit virtually superimposable survival responses at 43°C despite polyamine depletion. These data suggest a potential relationship between heat-inducibility of polyamine catabolism and heat sensitization by DFMO (see Discussion).

#### *Thermotolerance decay*

An appreciation of the expression and decay kinetics of thermotolerance is critical to the successful implementation of clinical hyperthermia programs (6). We have examined the expression and decay of thermotolerance in rodent and human cell lines using split heat dose experiments as depicted in Figure 6. While only minor variations in the time course for development of thermotol-

Table 1. Comparison of the DFMO-dependent heat-inducibility of N<sup>1</sup>-SAT activity and sensitization to hyperthermia-induced cytotoxicity by DFMO in cell cultures

Cell line	HS (temp. × time-min)	N <sup>1</sup> -SAT activity (units/10 <sup>6</sup> cells)			Sensitization by DFMO (% survival)*	
		-HS	+HS*		-DFMO	+DFMO
			-DFMO	+DFMO		
CHO	43° × 90	2.1 ± 0.1	21.7 ± 1.3	1.4 ± 0.2	2.96 ± 0.34	0.012 ± 0.0012
	45° × 30				0.11 ± 0.03	0.003 ± 0.001
A549	45° × 20	2.4 ± 0.3	16.4 ± 1.7	4.2 ± 0.3	0.08 ± 0.01	0.004 ± 0.0001
	45° × 40					
HeLa	43° × 90	0.5 ± 0.1	0.5 ± 0.1	—	5.34 ± 1.60	5.43 ± 2.15

\* N<sup>1</sup>-SAT activity was measured at 6 hr after these heat shocks. For survival experiments, cells were treated with DFMO (5 mM) for 8 hr and then incubated at 37°C for 36 hr prior to HS; for enzyme studies, the cultures were treated with DFMO (5 mM) continuously for 48 hr prior to HS.

HS = Heat shock; -HS = Non-heat shocked control.

erance are seen among the three cell lines examined, the decay pattern is markedly prolonged for CHO and A549 cells as compared with HeLa cells. Even at 60 hr after the initial heat stress, CHO and A549 cells are on the order of 2 logs more resistant to subsequent heating than controls. HeLa cells, on the other hand, demonstrate complete thermotolerance decay by 50 hr. The potential relationship between heat-inducibility of polyamine acetylation, and the maintenance of thermotolerance will be further discussed.

#### Hyperthermia/DFMO effects on human tumors

Human tumor cell clonogenic assays have been used to screen for the activity of anticancer agents toward a particular tumor. Table 2 depicts the response of human melanoma tumor cells, obtained from patient biopsy, to a battery of cytotoxic agents (Vinzolidine), biologic response modifiers (MTP,  $\alpha$ -IFN,  $\gamma$ -IFN, Interleukin-2), hyperthermia and hyperthermia plus DFMO combinations. Of the agents tested, DFMO followed by 42°C heating

afforded the largest cytotoxic effect as measured by colony formation. This combination resulted in more than a 50% reduction in colony forming ability under all conditions tested. In this assay system, such reductions in colony formation have been associated with a 20–30% rate of clinical tumor response (18). These results show that for this patient sample, the combination of 42°C hyperthermia and DFMO is the most effective of the agents tested in reducing melanoma cell colony formation.

#### Systemic hyperthermia

Figures 7 and 8 depict, respectively, the CDRH Helix used for induction of systemic hyperthermia, and a representative time versus temperature plot for a patient undergoing whole body hyperthermia. Previous and in progress publications describe in detail the hyperthermia physics and technology involved in the CDRH Helix for regional and systemic hyperthermia (10, 20, 21). However, one important feature of this heating method deserves highlighting in Figure 8. Note that the time to achieve "therapeutic" temperatures (defined here as  $\geq 41.5^\circ\text{C}$ ) is on the order of 25 minutes. This relatively rapid rate of core heating is critical to the avoidance of thermotolerance development which has presented a major biological stumbling block to the use of systemic hyperthermia in cancer therapy.

## DISCUSSION

This study investigates the biological rationale for combining systemic hyperthermia with polyamine biosynthesis inhibition in the treatment of human cancer. Figures 2–4 describe phenomena related to the heat stress-induced catabolism (acetylation and oxidation) of endogenous polyamines. The finding that heat shock stimulates acetylation of spermidine (Fig. 2), via activation of N<sup>1</sup>-SAT (9), led us to investigate the potential physiological consequences of polyamine catabolism. No biological purpose has yet been ascribed to this metabolic pathway.

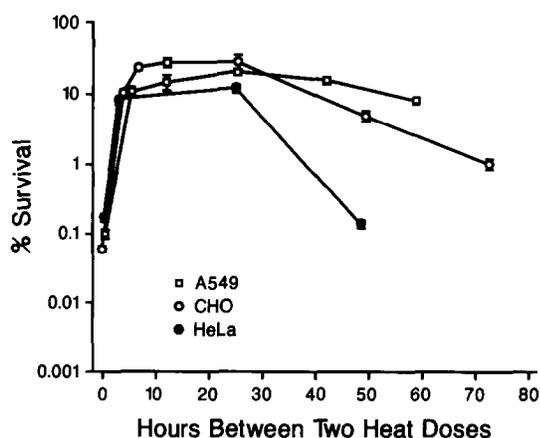


Fig. 6. Thermotolerance decay kinetics in CHO (○), HeLa (●), and A549 (□) cells measuring colony survival via split heat dose experiments. Heating sequences were; CHO (45°C × 10 min, then 20 min), HeLa (43°C × 60 min, then 120 min), A549 (45°C × 10 min, then 30 min).

Table 2. Survival, as measured by colony forming ability, of human melanoma tumor cells isolated from a tumor biopsy and grown in soft agar cultures in response to treatments with various anticancer agents

Agent tested	Duration	Concentration	Survival (%)*
Vinzolidine	1 hr	1 $\mu\text{g/ml}$	98.00 $\pm$ 18.07
Liposomal MTP	1 hr	40 $\mu\text{g/ml}$	112.25 $\pm$ 13.82
$\alpha$ -IFN	Continuous	4 ng/ml	76.02 $\pm$ 12.21
$\gamma$ -IFN	Continuous	1000 units	72.46 $\pm$ 3.81
Interleukin-2	Continuous	1000 units	86.71 $\pm$ 11.58
42°C	1 hr	0.5 mM	84.14 $\pm$ 10.12
DFMO	3 hr	5 mM	36.43 $\pm$ 5.00
DFMO $\rightarrow$ 42°C	DFMO 3 hr, Wait 48 hr, then 42°C	0.5 mM	66.12 $\pm$ 4.25
		5 mM	31.48 $\pm$ 2.17
			44.25 $\pm$ 1.22

\* Untreated colony forming efficiency was 0.13%.

The constitutive oxidation of acetylated spermidine by PAO liberates both acetamidopropanal (a reactive aldehyde) and  $\text{H}_2\text{O}_2$ . We initially hypothesized that these reactive oxygen species, generated from endogenous polyamine oxidation after heat stress, might be responsible in part for hyperthermic cytotoxicity. This hypothesis was tested by using the inhibitor of PAO to study cell survival responses in control cultures and in cultures depleted of endogenous glutathione which is known to function in the neutralization of free  $\text{H}_2\text{O}_2$  (13). These studies revealed that heat stress-induced polyamine catabolism leads to a reduction in cellular glutathione levels (Fig. 3). However, only a partial block of the glutathione reduction after heat shock is achieved with inhibition of polyamine oxidation suggesting that other endogenous defense systems, such as catalase, superoxide dismutase or nucleophilic protein moieties, may provide a majority influence in detoxifying these oxygen species.

Inhibition of polyamine oxidation has no discernible effect on cell survival responses unless cultures are pre-

viously depleted of endogenous glutathione pools (Fig. 4). Such results suggest that the byproducts of polyamine oxidation are not normally toxic to cells undergoing hyperthermic stress. In fact, since  $\text{N}^1$ -SAT is heat-inducible in the presence of polyamines, and polyamine depletion increases heat sensitivity, we infer that polyamine acetylation may afford some protection against the toxic effects of heat stress. This conclusion leads to the hypothesis that inhibitors of polyamine acetylation may also serve as potent sensitizers of heat shock responses.

Not all cell lines studied were found to exhibit thermal sensitization after polyamine depletion with DFMO (Fig. 5). Such exceptions led us to reinvestigate three distinct phenomena regarding the interactions of polyamine metabolism and hyperthermia across several cell lines; specifically, the heat-inducibility of polyamine acetylation (Table 1), the heat sensitization afforded by polyamine depletion (Table 1), and finally the expression and subsequent decay of thermotolerance using split heat dose experiments (Fig. 6). A striking pattern emerges revealing

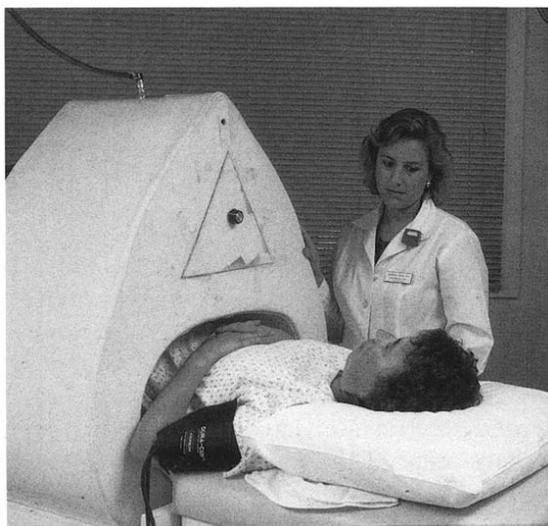


Fig. 7. CDRH Helix with model patient in position for systemic hyperthermia treatment.

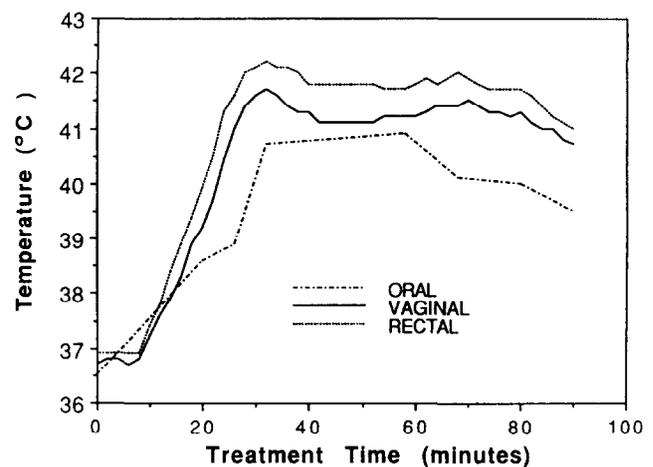


Fig. 8. Temperature versus time plot of oral, vaginal and rectal temperatures in a patient with metastatic melanoma receiving systemic hyperthermia in conjunction with oral DFMO.

that cells exhibiting heat-inducible polyamine acetylation are sensitized to heat by DFMO, and ultimately display a prolonged decay pattern of thermotolerance (CHO and A549). Conversely, HeLa cells display non heat-inducible polyamine acetylation, are not sensitized to heat with DFMO, and demonstrate more rapid kinetics of thermotolerance decay.

Interpretation of such correlative observations requires reexamination of the overall scheme of polyamine metabolism (Fig. 1). The induction of N<sup>1</sup>-SAT by heat stress appears to require spermidine and putrescine as evidenced by abolishment of this induction in cultures depleted of spermidine and putrescine by DFMO (Table 1). The finding that HeLa cells do not display heat-inducible polyamine acetylation may merely reflect a lesser availability of cycling spermidine and putrescine pools. A putative function of endogenous polyamines is that their linear structure and protonated state at physiological pH affords general buffering and stabilizing functions toward negatively charged proteins and DNA molecules within the cell (24). Cell lines with large pools of unconjugated or "bioavailable" polyamines might therefore be more susceptible to polyamine depletion by DFMO and subsequent sensitization by hyperthermic stress. HeLa cells may more effectively compartmentalize or sequester their intracellular polyamines in association with critical targets, thus the absence of heat sensitization when cycling pools are interrupted by DFMO.

The concept of compartmentalization of intracellular polyamines was originally advanced by Dewey (5), and might be expanded to evaluate the thermotolerance decay patterns depicted in Figure 6. If cycling or bioavailable pools of polyamines can provide molecular stabilization functions after heat stress, their presence may affect patterns of thermotolerance decay. Recent work in the A549 cell line has demonstrated that the decay of thermotolerance can be rapidly accelerated by prior polyamine depletion with DFMO (17). Future studies are underway to clarify the role of endogenous polyamines in thermotolerance responses.

The potency of hyperthermic enhancement via polyamine depletion *in vitro* has encouraged its evaluation in the clinic. A Phase I trial has therefore been initiated at

the University of Arizona Cancer Center using oral DFMO in conjunction with systemic hyperthermia in the treatment of metastatic melanoma. Melanoma cultures from human tumor biopsies demonstrate potent heat sensitization following polyamine depletion by DFMO. Previous clinical and *in vitro* studies have indicated modest activity of DFMO alone (1, 13), and in combination with other anticancer agents (2, 4, 22). The data presented in this paper would suggest that further therapeutic gains might be achieved by combining the cytostatic effects of polyamine biosynthesis inhibition (DFMO), with the tumoricidal activity of hyperthermia.

We have confirmed *in vitro* that hyperthermic enhancement with DFMO can be achieved at 42°C (unpublished results—D. J. M. Fuller, May 1986) which represents a more feasible target temperature for whole body heating. To date, 24 systemic hyperthermia treatments have been delivered to nine patients in conjunction with oral DFMO administration under the guidance of a Phase I clinical trial for treatment of metastatic melanoma. Oral DFMO is administered every 8 hr for 4 days with hyperthermia delivered on day 6. This course is repeated every 2 weeks for 3–4 cycles with the planned dose escalation ranging from 0.5 gm/m<sup>2</sup> Q8 hr - 5.0 gm/m<sup>2</sup> Q8 hr.

A critical element in achieving hyperthermic sensitization by DFMO involves the actual depletion of endogenous polyamines in the cells of interest (7, 8, 16). Tissues or compartments which do not verifiably demonstrate depletions of putrescine and spermidine, would not be expected to display heat sensitization. We therefore perform HPLC analysis of polyamine levels obtained from skin biopsies and buccal mucosa smears on patients being treated with DFMO as part of the Phase I protocol. Such evaluation will be critical in documenting that the oral administration of DFMO can successfully deplete endogenous polyamine pools not only in GI mucosa, but in epidermis and subcutaneous tissue as well. Studies reporting on both the technology involved in achieving whole body hyperthermia, and the complete Phase I toxicity analysis are in progress. We continue aggressive efforts aimed at translating intriguing and reproducible findings from the cancer biology laboratory into the clinic in the treatment of human malignancy.

## REFERENCES

1. Abeloff, M. D.; Rosen, S. T.; Luk, G. D.; Baylin, S. B.; Zeltzman, M.; Sjoerdsma, A. Phase II trials of  $\alpha$ -difluoromethylornithine, an inhibitor of polyamine synthesis, in advanced small cell lung cancer and colon cancer. *Cancer Treat. Rep.* 70:843–845; 1986.
2. Arundel, C. M.; Nishioka, K.; Tofilon, P. J. Effects of  $\alpha$ -difluoromethylornithine-induced polyamine depletion on the radiosensitivity of a human colon carcinoma cell line. *Radiat. Res.* 114:634–640; 1988.
3. Bey, P.; Bolkenius, F. N.; Seiler, N.; Casara, P. N-2,3-butadienyl-1,4 butanediamine derivatives: potent irreversible inactivators of mammalian polyamine oxidase. *J. Med. Chem.* 28:1–2; 1985.
4. Croghan, M. K.; Booth, A.; Meyskens, F. L. A phase I trial of recombinant interferon- $\alpha$  and  $\alpha$ -difluoromethylornithine in metastatic melanoma. *J. Biol. Response Mod.* 7:409–415; 1988.
5. Dewey, W. C. Interaction of heat with radiation and chemotherapy. *Cancer Res.* 44:4714–4720; 1984.
6. Field, S. B. Clinical implications of thermotolerance. In: Overgaard, J., ed. *Hyperthermic oncology*, Vol. 2. London: Taylor & Francis; 1984:235–244.
7. Fuller, D. J. M.; Gerner, E. W. Delayed sensitization to heat by inhibitors of polyamine-biosynthetic enzymes. *Cancer Res.* 42:5046–5049; 1982.
8. Fuller, D. J. M.; Gerner, E. W. Sensitization of Chinese

- hamster ovary cells to heat shock by  $\alpha$ -difluoromethylornithine. *Cancer Res.* 47:816–820; 1987.
9. Harari, P. M.; Fuller, D. J. M.; Gerner, E. W. Heat shock stimulates polyamine oxidation by two distinct mechanisms in mammalian cell cultures. *Int. J. Radiat. Oncol. Biol. Phys.* 16:451–457; 1989.
  10. Harari, P. M.; Shimm, D. S.; Gerner, E. W.; Alberts, D. S. Intraperitoneal chemotherapy plus regional/systemic hyperthermia in the treatment of advanced ovarian cancer. *Reg. Cancer Treat.* 2:54–58; 1989.
  11. Libby, P. R. Calf liver nuclear N-acetyltransferases. *J. Biol. Chem.* 253:233–237; 1978.
  12. Matsui, I.; Wiegand, L.; Pegg, A. E. Properties of spermidine N-acetyltransferase from livers of rats treated with carbon tetrachloride and its role in the conversion of spermidine into putrescine. *J. Biol. Chem.* 256:2454–2459; 1981.
  13. Meister, A.; Anderson, M. A. Glutathione. *Ann. Rev. Biochem.* 52:711–760; 1983.
  14. Meyskens, F. L.; Kingsley, E. M.; Glatke, T.; Loesch, L.; Booth, A. A phase II study of  $\alpha$ -difluoromethylornithine (DFMO) for the treatment of metastatic melanoma. *Investig. New Drugs* 4:257–262; 1986.
  15. Mitchell, J. B.; Russo, A. Thiols, thiol depletion and thermosensitivity. *Radiat. Res.* 95:471–485; 1983.
  16. Pegg, A. E. Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. *Cancer Res.* 48:759–774; 1988.
  17. Roizin-Towle, L.; Yarlett, N.; Hall, E. J.; Pirro, J.; Bayne, G.; Delohery, T. Inhibition of polyamine biosynthesis and hypoxic cell radiosensitization in human lung carcinoma cells (Abstr. 2–10). *Chem. Mod. of Cancer Treat. VI<sup>th</sup> Conference*; 1988.
  18. Salmon, S. E.; Hamburger, A. W.; Soehnen, B.; Durie, B. G.; Alberts, D. S.; Moon, T. E. Quantitation of differential sensitivity of human-tumor stem cells to anticancer drugs. *N. Engl. J. Med.* 298:1321–1327; 1978.
  19. Seiler, N.; Knodgen, B. High-performance liquid chromatographic procedure for the simultaneous determination of the natural polyamines and their monoacetyl derivatives. *J. Chromatog.* 221:227–235; 1980.
  20. Shimm, D.; Cetas, T.; Hynynen, K.; Buechler, D.; Anhalt, D.; Aristizabal, S.; Sykes, H.; Cassady, J. The CDRH helix: clinical thermal dosimetry. *Am. J. Clin. Oncol.* 12:110–113; 1989.
  21. Shimm, D.; Cetas, T.; Oleson, J.; Cassady, J.; Sim, D. Clinical evaluation of hyperthermia equipment. The University of Arizona institutional report for the NCI Hyperthermia Equipment Evaluation Contract. *Int. J. Hyperther.* 4:39–51; 1988.
  22. Sunkara, P. S.; Prakash, N. J.; Mayer, G. D.; Sjoerdsma, A. Tumor suppression with a combination of  $\alpha$ -difluoromethylornithine and interferon. *Science* 219:851–853; 1983.
  23. Sunkara, P. S.; Rosenberger, A. L. Antimetastatic activity of DL- $\alpha$ -difluoro-methylornithine, an inhibitor of polyamine biosynthesis, in mice. *Cancer Res.* 47:933–935; 1987.
  24. Tabor, C. W.; Tabor, H. Polyamines. *Ann. Rev. Biochem.* 53:749–790; 1984.
  25. Tietze, F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: application to mammalian blood and other tissues. *Anal. Biochem.* 27:502–522; 1969.