Eur J Cancer Clin Oncol, Vol. 23, No. 2, pp. 201–204, 1987.
Printed in Great Britain

0277-5379/87$3.00+0.00

Effect of Hyperthermia on Spermidine/Spermine N^1-Acetyltransferase Activity in Ehrlich Ascites Cells

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Abstract—*When Ehrlich ascites tumor cells were incubated at 42° C, the activity of spermidine/spermine N^1-acetyltransferase, a rate-limiting enzyme of polyamine biodegradation, decreased to 20% of the control level within 1 hr. Protein synthesis, judged from the incorporation of [^3H]leucine into the acid-insoluble fraction, was less affected by heat exposure. The decrease in the enzyme activity caused by heat treatment could be reversed by switching the incubation temperature back to 37° C. The reversion of enzyme activity was suppressed by cycloheximide but not by actinomycin D.*

INTRODUCTION

HYPERTHERMIA might be used to treat cancer clinically either alone or in combination with radiation or chemotherapy [1, 2]. Exposure of cells in culture to temperature above 41° C results in a loss of their ability to proliferate [3, 4]. Neoplastic and transformed cells may be more sensitive to thermal killing than normal cells [5, 6].

Putrescine, spermidine, and spermine are organic cations widely distributed in eukaryotic cells. These polyamines seem to be important in cell growth and tumor promotion [7, 8]. Changes in polyamine biosynthesis in response to hyperthermia has been studied in Chinese hamster cells. When such cells were exposed to hyperthermia of 43° C, intracellular polyamine levels decreased and extracellular levels increased [9]. Verma and Zibell [10] reported that induction of mouse epidermal ornithine decarboxylase, a rate-limiting enzyme in polyamine biosynthesis, was inhibited by incubation at high temperatures both *in vitro* and *in vivo*. However, exogenous polyamines enhance cell killing by hyperthermia [11]. The biochemical nature of hyperthermia-induced changes of polyamine metabolism is still poorly understood.

Exposure to a number of stimuli causes a large change in the activity of spermidine/spermine N^1-acetyltransferase in rat liver and kidney and also in bovine lymphocytes. This enzyme is a rate-limiting enzyme in polyamine biodegradation pathway [12–15]. In the present study, we investigated the effect of hyperthermic treatments on spermidine/spermine N^1-acetyltransferase activity in Ehrlich ascites cells.

Accepted 30 July 1986.
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MATERIALS AND METHODS

Cell culture

Ehrlich ascites cells were maintained by sequential transplantation in female ICR mice (Schizuoka Experimental Animal Cooperative Society, Shizuoka). The cells were recovered, washed, suspended in Eagle's minimum essential medium (Nakarai Chemicals, Kyoto) containing 10% fetal calf serum (M.A. Bioproducts, Wakerville, MD) and 10% dimethyl sulfoxide, and stored at −80° C until *in vitro* culture. After being washed, Ehrlich ascites cells were suspended in Eagle's minimum essential medium containing 10% fetal calf serum and cultured in a humidified atmosphere of 5% CO_2 in air at 37° C for 2 days. Then, cells in a high-walled Petri dish (45 mm × 70 mm) were heated at 37° C or 42° C in a temperature-controlled water bath in a CO_2 incubator.

Assay of spermidine/spermine N^1-acetyltransferase activity

Preparation of the enzyme solution from cells was as described previously [16]. Spermidine/

spermine N^1-acetyltransferase activity was assayed by following the incorporation of [acetyl-1-^{14}C]acetyl CoA (55.0 mCi/mmol, New England Nuclear, Boston, MA) into monoacetylspermidine [12]. Protein was measured by the method of Lowry *et al.* [17] using bovine serum albumin as the standard.

Assay of [^{3}H]leucine incorporation into acid-insoluble fraction

Cells were labeled for 1 hr at 37°C or 42°C with 1 μCi of [^{3}H]leucine (58.4 mCi/mmol, New England Nuclear) in each dish. Then, cells were washed 2 times with phosphate-buffered saline, suspended in cold 5% trichloroacetic acid, and kept on ice for 30 min. The pellet obtained by centrifugation was dissolved in 1 ml of 0.1 N NaOH. Portions of the solution were used for assays of radioactivity and protein concentration.

Assay of [^{3}H]thymidine incorporation into acid-insoluble fraction

[^{3}H]Thymidine incorporation into DNA was assayed as the incorporation of [^{3}H]thymidine into the acid-insoluble fractions. After hyperthermic treatments, cultures were labeled for 20 hr at 37° C with 0.5 μCi of [6-^{3}H]thymidine (19.3 mCi/mmol, New England Nuclear) per dish. After incubation, cells were placed on Whatman glass filter GF/B. The filters were washed with 5% trichloroacetic acid, dried and placed in counting vials containing 5 ml of a toluene-based scintillator (0.4% DPO, 0.01% POPOP) and radioactivity was measured.

RESULTS

When the cells were exposed to incubation at 42° C, the activity of spermidine/spermine N^1-acetyltransferase rapidly decreased. Enzyme activity decreased by 80% after 45 min of treatment (Table 1). Under the same condition, we measured the effect of hyperthermic treatment on DNA synthesis. The DNA synthesis in cells exposed to 42° C for 1 and 2 hr decreased to 53 and 21%, respectively (Table 2). These results suggest that the decrease in enzyme activity has a link with the alteration of DNA synthesis. To see whether the decrease in enzyme activity by heat treatment is due to inhibition of protein synthesis in general, the incorporation of [^{3}H]leucine into the acid-insoluble fraction was measured. When cells were incubated at 42° C for 1 hr, the decrease in protein synthesis was small (20%), suggesting that incubation at 42° C affected the synthesis of some proteins including spermidine/spermine n^1-acetyltransferase (Table 3).

Cell cultures were exposed to 42° C for 1 hr and then incubated at 37° C for up to 4 hr. The hyperthermia-induced inhibition of spermidine/spermine N^1-acetyltransferase was reversed, and

Table 1. Effect of hyperthermia on spermidine/spermine N^1-acetyltransferase activity in Ehrlich ascites cells

Treatment*	Spermidine/spermine N^1-acetyltransferase activity pmol/mg protein/10 min	% of control
Control	659†	100
42° C, 10 min	579	88
42° C, 30 min	493	75
42° C, 45 min	135	20
42° C, 1 hr	124	19
42° C, 2 hr	89	14

*Cells were cultured at 37° C for 2 days and then exposed to 42° C for the times shown.
†Values are means of two assays. Similar results were obtained in 2 additional experiments.

Table 2. Effect of hyperthermia on [^{3}H]thymidine incorporation into acid-insoluable fraction

Treatment	[^{3}H]Thymidine incorporation into acid-insoluble fraction cpm/2 × 10^5 cells	% of control
37° C (control)	13632 ± 704*	100
42° C, 1 hr	7146 ± 579	53.0
42° C, 2 hr	2883 ± 278	21.1

After hyperthermic treatments, cells were labeled for 20 hr at 37° C with 0.5 μCi of [^{3}H]thymidine in each dish.
*Values are the means ± S.E. for three experiments.

Table 3. Effect of hyperthermia on incorporation of [^{3}H]leucine into acid-insoluble fraction

Treatment*	[^{3}H]Leucine incorporated into acid-insoluble fraction cpm/mg protein	% of control
37° C, 1 hr	3950 ± 86†	100
42° C, 1 hr	3176 ± 28	80

*Cells were cultured at 37° C for 2 days and then exposed to 37 or 42° C. Cells were labeled for 1 hr with 1 μCi of [^{3}H]leucine in each dish.
†Values are means ± S.E. for three experiments.

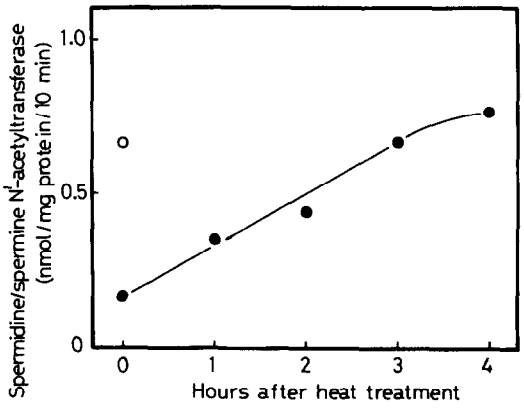

Fig. 1. Recovery of spermidine/spermine N^1-acetyltransferase activity after hyperthermia. Cells were cultured at 37° C for 2 days and then exposed to 42° C for 1 hr. Thereafter, the cells were incubated at 37° C for up to 4 hr. ○, control cells; ●, cells exposed to 42° C.

the enzyme activity reached the control level 3 hr after incubation at 37° C (Fig. 1). These results suggested this enzyme was sensitive to thermal inactivation and that the inhibition of the enzyme activity by hyperthermic treatment was reversible. To learn more about the mechanism of inhibition, we tested the effect of cycloheximide and actinomycin D on the recovery of enzyme activity from inhibition. Recovery was suppressed by cycloheximide but not by actinomycin D (Table 4). These results suggested that the synthesis of newly-formed RNA was not required for the recovery of spermidine/spermine N^1-acetyltransferase activity.

DISCUSSION

Our results showed that spermidine/spermine N^1-acetyltransferase in Ehrlich ascites cells was suppressed by hyperthermic treatment. The conversion of the polyamines, spermine and spermidine back to putrescine involves sequential actions of two enzymes, spermidine/spermine N^1-acetyltransferase and polyamine oxidase [12–16, 18]. The first of these enzymes is a rate-limiting enzyme in the acetylase/oxidase pathway [13, 14]. Another major pathway of putrescine formation is that of polyamine biosynthesis from ornithine [7, 8]. In this pathway, ornithine decarboxylase is a rate-limiting enzyme. Verma and Zibell [10] have reported that induction of mouse epidermal ornithine decarboxylase by phorbol myristate acetate is inhibited by incubation at high temperatures *in vivo* and *in vitro*. Ornithine decarboxylase activity also decreased by 40% after 1 hr of heat treatment under our experimental conditions (data not shown). These results, together with ours, suggest

Table 4. Effect of cycloheximide and actinomycin D on recovery of spermidine/spermine N^1-acetyltransferase activity

Treatment*	Spermidine/spermine N^1-acetyltransferase activity pmol/mg protein/10 min	% of control
37° C (control)	652	100
42° C, 1 hr	160	25
42° C, 1 hr — 37° C, 3 hr	661	101
42° C, 1 hr — actinomycin D + 37° C, 3 hr	587	90
42° C, 1 hr — cycloheximide + 37° C, 3 hr	185	28

*Cells were cultured at 37° C for 2 days and then exposed to 42° C for 1 hr. Thereafter, cycloheximide (20 μg/ml) or actinomycin D (5 μg/ml) was added to the culture medium and the cells were incubated at 37° C for 3 hr.

that hyperthermic treatment depletes intracellular putrescine. Fuller and Gerner [19] reported that putrescine can rescue cells from the effect of an irreversible inhibitor of ornithine decarboxylase, difluoromethylornithine which causes increased sensitivity to hyperthermia. Thus, a decrease in the concentration of putrescine may be involved in hyperthermia-induced cytotoxity.

Our findings also demonstrated that the inhibition of spermidine/spermine N^1-acetyltransferase activity by hyperthermia could be reversed by switching the incubation temperature to 37° C, and that the reversion of the enzyme activity was suppressed by cycloheximide but not by actinomycin D. These results mean that the synthesis of new RNA was not needed for recovery of the enzyme activity, suggesting that hyperthermic treatment has no effect on messenger RNA levels and mainly affects translational levels of spermidine/spermine N^1-acetyltransferase synthesis.

Acknowledgements—The authors wish to thank Miss C. Latta for her critical reading of the manuscript.

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