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HYPERTHERMIA AND POLYAMINE BIOSYNTHESIS: DECREASED ORNITHINE DECARBOXYLASE INDUCTION IN SKIN AND KIDNEY AFTER HEAT SHOCK[1]

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Summary: The effect of hyperthermia treatments on ornithine decarboxylase (ODC) induction in mouse tissue was determined both in vitro and in vivo. In vitro, the addition of 12-O-tetradecanoylphorbol-13-acetate (TPA) to adult mouse skin pieces incubated at 37°C in serum-free MEM led to a dramatic increase in epidermal ODC activity 5 hours following treatment. In contrast, incubation temperatures of 40°C for the entire 5 hour incubation period rendered the skin pieces unresponsive to TPA for ODC induction. This inhibition of ODC induction was not the result of thermal skin kill, inactivation of TPA, or a general effect on epidermal protein synthesis. The inhibition of ODC induction could be reversed by switching the incubation temperature back to 37°C. In vivo, raising the core body temperature in male mice to 41°C for 1 hour resulted in a 78% decrease in kidney ODC activity. The kidney DNA synthesis and protein synthesis remained unaltered following the whole body hyperthermia treatments.

Hyperthermia has been shown to be a potential clinically applicable antineoplastic treatment when used either alone or in combination with radiation treatments or chemotherapeutic agents. Data from studies with cultured mammalian cells and with animal tumor model systems indicate that hyperthermia augments the cytocidal effects of these other cancer treatment modalities (1-7). Evidence also suggests that neoplastic and transformed cells may be more sensitive to thermal killing than normal cells (8-10).

The biochemical mechanism of action of hyperthermia treatments is unclear (11-18). The available evidence suggests that the induction of mouse epidermal ODC activity by the potent skin tumor promotor TPA may be an essential

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component of the mechanism of tumor promotion by TPA (19,20). A number of agents such as certain retinoids and α-difluoromethylornithine (DFMO), a suicide inhibitor of ODC, which block TPA-induced ODC activity, also inhibit TPA-induced skin tumors (20,21).

ODC, which decarboxylates ornithine to putrescine, is the first and the rate-limiting enzyme in the pathway of mammalian polyamine biosynthesis (22,23). Increased ODC activity and the subsequent accumulation of polyamines are associated with increased cell proliferation, altered patterns of differentiation, and the tumor phenotype (23). Because of the potential significance of ODC as a target for a number of agents used for cancer prevention and/or treatment (24,25), we investigated the effects of hyperthermia on TPA-mediated induction of mouse epidermal ODC activity *in vitro*, and the effects of whole body hyperthermia treatments on the level of ODC activity in male mouse kidneys, an organ that has been shown to have the highest level of ODC activity in the mouse. The supporting data that inhibition of ODC induction by hyperthermia treatment may be one of its possible mechanisms for anti-cancer effect are summarized in this communication.

Materials and Methods

Female Charles River CD-1 mice, 7-9 weeks of age, were housed and treated as previously described (21). Male ICR mice were purchased from Harlan Sprague Dawley, Madison, WI. TPA was purchased from Life Systems, Newton, MA. DL-[1-^{14}C]-ornithine hydrochloride (specific activity, 49.9 mCi/mmol), L-[4-5-^{3}H]-leucine (specific activity, 50 Ci/mmol), and [methyl-^{3}H]-thymidine (specific activity, 20 Ci/mmol) were purchased from New England Nuclear.

For the *in vitro* experiments, skin was excised from the shaved backs of the female CD-1 mice following cervical dislocation. The skin pieces were incubated at the desired temperatures for 5 hours in serum-free MEM for ODC induction by TPA as previously described (26). For protein synthesis and DNA synthesis, [^{3}H]-leucine or [^{3}H]-thymidine was added to the solution to a final concentration of 10 μCi/ml for the last one hour of incubation. After the incubation period, incorporation of [^{3}H]-thymidine and [^{3}H]-leucine into epidermal DNA and protein respectively was determined (27).

For the *in vivo* experiments, male ICR mice were injected i.p. with 1 ml isotonic saline, then heated in a radiant hyperthermia chamber so that a core body temperature of 41°C was maintained for one hour using the techniques of Robins *et al.* (28), and using his apparatus. Following the 1 hour exposure to whole body hyperthermia, the mice were killed, the kidneys were excised, perfused, and the soluble kidney extract was prepared for ODC assays (26). For the DNA incorporation trials, the mice were injected i.p. with an additional 0.2 ml (1 μCi/gm body wt.) [^{3}H]-thymidine in saline (0.9% NaCl) immediately after the hyperthermia treatment and sacrificed 30 minutes later (27).

ODC activity in the soluble epidermal extract was determined by measuring the release of $^{14}CO_2$ from DL-[1-^{14}C]-ornithine hydrochloride as described (21). DNA content of the homogenates was measured by the diphenylamine method of Burton (29), and the protein content of the soluble extracts was measured by the procedure of Lowry et al. (30).

Results

In vitro experiments: We have previously shown that adult mouse skin pieces remain responsive to ODC induction by TPA when incubated in serum-free MEM. Addition of TPA to the incubated skin pieces results in a dramatic increase in ODC activity, peaking at 6 hours, and returning to basal levels by 18 hours (26). The effect of a 40°C incubation temperature on ODC induction by TPA is shown in Table 1. The TPA induced rise in ODC activity was affected by the 40°C incubation temperature. A 66% decrease in ODC activity was noted after 1 hour of the 5 hour incubation was executed at 40°C, and a 93% decrease was observed after a full 5 hour treatment (see Table 1).

The inhibition of ODC induction by TPA in skin pieces exposed to a 40°C incubation temperature was reversible. In this experiment, skin pieces were incubated for 5 hours at 40°C without TPA, then TPA was added and the skin pieces were incubated for another 5 hours at 37°C. ODC activity was induced to the same amount as in the control (see Figure 1).

This lack of ODC induction by TPA at 40°C was observed not to be due to inactivation of TPA. In this experiment, skin pieces were incubated at either 37°C or 40°C in the presence of TPA as usual. After the 5 hour incubation, the skin pieces were removed and discarded, and fresh pieces were added.

Table 1. The Effect of a 40°C Incubation Temperature on TPA Induction of ODC Activity in Incubated Mouse Skin Explants

	Incubation time (hrs) at:		ODC activity (nmol CO_2/60 min/mg protein)	
	37°C	40°C	ethanol	TPA
Experiment 1	5	0	0.15 ± .08	2.21 ± .73
	0	5	.07 ± .05	.07 ± .02
Experiment 2	5	0	-	1.37 ± .65
	4	1	-	.46 ± .09
	0	5	-	.10 ± .03

Mouse skin pieces were incubated at the indicated temperatures for a total of 5 hours in MEM in the presence of 2.5 μM TPA in ethanol or ethanol alone. Each value is the mean ± S.E. of determinations carried out on 6 skin pieces from 3 mice.

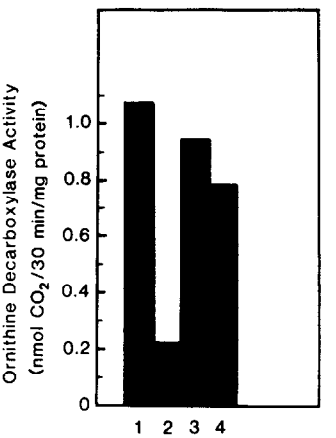

Figure 1. Test of Skin Viability at 40°C

1. Skin pieces were incubated at 37°C for 5 hours in the presence of TPA.
2. Skin pieces were incubated at 40°C for 5 hours in the presence of TPA.
3. Skin pieces were incubated at 37°C for 5 hours in the absence of TPA, then TPA was added to the medium and they were incubated an additional 5 hours at 37°C.
4. Skin pieces were incubated at 40°C for 5 hours in the absence of TPA, then TPA was added to the medium and they were incubated an additional 5 hours at 37°C.

All values are the average of the determinations of 6 skin pieces from 3 mice.

These were all incubated in the preconditioned medium for 5 hours at 37°C. ODC activity in the skin pieces incubated in the medium that had been preconditioned at 37°C was 0.70 nmole CO_2/60 min/mg protein. ODC activity in the skin pieces incubated in the medium that had been preconditioned at 40°C was 0.76 nmole CO_2/60 min/mg protein.

As shown in Table 2, a 40°C incubation temperature affected neither [^{3}H]-leucine nor [^{3}H]-thymidine incorporation into mouse epidermal protein or DNA.

Table 2. The Effect of 40°C Incubation Temperature on the Incorporation of Tritiated Precursors into Epidermal Protein and DNA

Incubation temperature	[^{3}H]-leucine incorporation (dpm/µg protein)	[^{3}H]-thymidine incorporation (dpm/µg DNA)
37°C	10.3 ± 1.0	217 ± 27
40°C	9.1 ± 2.5	202 ± 43.5

Skin pieces were incubated for 5 hours at either 37°C or 40°C in serum-free MEM. Four hours into the incubation, either [^{3}H]-leucine or [^{3}H]-thymidine was added to a final concentration of 10 µCi/ml. After one hour, the incubation was stopped. Each value is the mean ± S.E. of the determinations from 6 skin pieces from 3 mice.

Table 3. The Effect of Whole Body Hyperthermia Treatments on Male Mouse Kidney ODC Activity and DNA Synthesis

	Core Temperature	ODC Activity (nmole CO_2/30 min/mg protein)	[^{3}H]-Thymidine incorporation (dpm/µg DNA)
Experiment I	38.43	12.26 ± 2.8	-
	41.03	2.83 ± 1.7	-
Experiment II	38.8	6.02 ± 3.3	37.12 ± 9.8
	40.7	1.32 ± 0.5	30.75 ± 5.3

Groups of six mice were exposed to whole body hyperthermia treatments resulting in a core temperature of approximately 41°C for 1 hr as described by Robins, et al. (28). Temperatures were measured by rectal probe every ten minutes. Identical controls were not heated. ODC activity was measured in homogenized kidney extract as previously described (21). DNA synthesis was measured by the amount of tritiated thymidine uptake, as described previously (27). Each value is the mean ± the S.E. of determinations carried out on six mice.

In vivo experiments: In this series of experiments, groups of six mice were exposed to whole body hyperthermia (core temperature of 41°C for 1 hour as measured by rectal probe). Control mice were kept at room temperature. The kidney ODC activity was determined either immediately after the hyperthermia treatment, or the mice were injected intraperitoneally with [^{3}H]-thymidine (1 µCi/gm. body wt.) in 0.2 ml of saline solution. The mice were then killed 30 minutes after the tritiated precursor injection, and the kidneys were excised, perfused, and homogenized in ice-cold distilled water. Aliquots were taken for determination of ODC activity and for measurement of [^{3}H]-thymidine uptake into DNA. The effects of whole body hyperthermia treatments on the level of ODC activity and DNA synthesis are shown in Table 3. A 41°C core temperature for 1 hour resulted in an inhibition of about 78% of the kidney ODC activity, but no measurable decrease in the rate of DNA synthesis.

Discussion

The data presented here demonstrates the thermal lability of ODC, the first enzyme in mammalian polyamine biosynthesis (22,23). Inhibition of ODC activity due to hyperthermic treatments was observed in both in vitro (Tables 1 and 2) and in vivo (Table 3) models. Inhibition of ODC induction in vitro does not appear to be the result of epidermal cell kill, because inducibility

was regained upon discontinuing the hyperthermic incubation temperatures (Figure 1). Also, there was no observed change in TPA stability, general protein synthesis, or general DNA synthesis under hyperthermic conditions.

Whole body hyperthermia treatments (core temperature of 41°C for 1 hour) in male mice also decreased kidney ODC activity (Table 3). We used male mouse kidney, because this organ has been shown to have the highest level of ODC activity in mice (31); thus effects could be easily observed. Again, hyperthermia treatments did not inhibit normal DNA synthesis (Table 3).

Alteration in polyamine biosynthesis in response to hyperthermia has been studied in Chinese hamster ovary cells (CHO). A 43°C hyperthermia exposure to the CHO cells resulted in decreased intracellular polyamine levels with concomitant increased extracellular levels (14). Furthermore, exogenous polyamines have been shown to enhance hyperthermia-induced cell killing (13). The mechanism of hyperthermia-induced cytotoxicity is not clearly understood (14, 17,32). Heat shock depresses DNA replication (14), causes increased levels of nuclear non-histone proteins, and may alter cell membrane functions (13).

Inhibition of ODC induction and the subsequent accumulation of putrescine have been correlated with the inhibition of the promotion of skin tumor formation by TPA (20,21). DFMO, a suicide inhibitor of ODC, has been shown to sensitize CHO cells to 43°C hyperthermia-mediated effects (15). Thus, we conclude that inhibition of ODC induction may be included as one of the complex mechanisms behind hyperthermia as a cancer treatment modality (11-18,33).

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