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Kinetics of Thermotolerance Induced in Mouse FM3A Cells and Its Suppression by Drugs

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Abstract: One of the biological problems of hyperthermia in its application to cancer treatment is thermotolerance, or transient thermoresistance in cell killing. Thermotolerance is induced by fractionated heating, by step-up heating and even during successive heating. In clinical hyperthermia thermotolerance will reduce therapeutic effectiveness. Kinetics of thermotolerance induced in mouse FM3A cells by fractionated heating and by step-up heating was analyzed in this study. Cepharanthin, as a modifier of cell membrane, and cisplatin, an anti-tumor drug, were examined if these drugs suppress the thermotolerance. It was found that these drugs suppressed thermotolerance in processes of the development and the expression of the induction of thermotolerance. As these drugs effectively suppressed the induction of hyperthermia, they can be beneficially used in combination with hyperthermia to improve therapeutic results.

Introduction

Hyperthermia has been examined experimentally and applied clinically as a new modality of cancer treatment.[1)] Hyperthermia has advantageous characteristics which is not known in conventional cancer treatments. Some cancer cells are more thermosensitive than normal cells, because of their inherent thermosensitiveness[2)] and of their conditional alteration of thermosensitiveness by low pH in circumstances of cancer cells.[3,4)] As temperature of cancer tissues is more easily elevated than that of normal tissues because of their deficient blood flow,[1)] the normal tissues will be protected from thermal damage. Hyperthermia is also applied in combination with chemotherapy[5-16)] and radiotherapy for thermoenhancement of these modalities.[17,18)] The problem which should be overcome in hyperthermia is thermotolerance which will decrease therapeutic effectiveness. Thermotolerance is experimentally induced by fractionated heating,[19-27)] step-up heating[29,30)] and during succesive heating[31-33)]. The thermotolerance would be also induced during a hyperthermic treatment or during a course of the treatments. In the present study, the kinetics of induced thermotolerance was analyzed, and drugs were applied in combination with hyperthermia to suppress thermotolerance.

Materials and Methods

The cells used were FM3A cells[34-36)] which were derived from a spontaneous mammary carcinoma in C3H mouse and which were established as a cell line cultured in suspension. Cells were cultured in Eagle's minimum essential medium (Nissui pharmaceutical Co., Tokyo) supplemented with 60*ug*/ml kanamycin and with 5% bovine calf serum (Hyclone Laboratories, Inc., Logan) and 5% fetal bovine serum (Hyclone Laboratories, Inc., Logan). Cells in test tubes were immersed in a water bath for the hyperthermic treatment at temperatures controlled within 0.1 ℃ .

Time courses for the induction of thermotolerance by fractionated heating and step-up heating are illustrated in Fig. 1. For fractionated heating (Fig. 1-a), the first heating was performed at 44 ℃ for 15 minutes (period-A) followed by the interval for varied time from 0 to 6 hours at 37 ℃ (period-B). And cells were challenged with the second heating at 44 ℃ for the assay of the surviving fraction (period-C). For step-up heating (Fig. 1-b), the first heating,

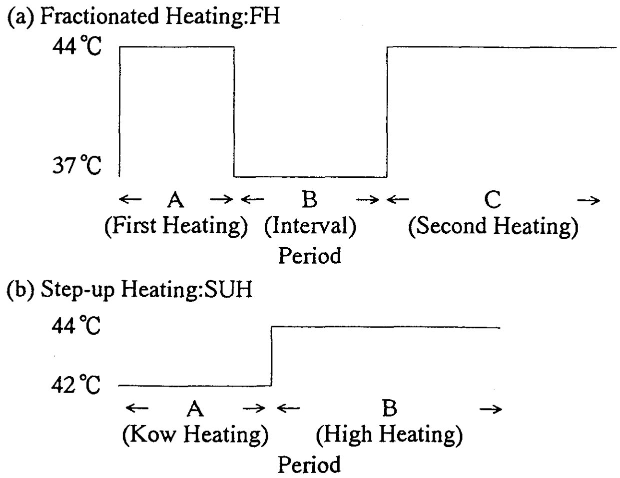


Fig. 1. Time course for the induction of thermotolerance.
(a) fractionated heating: Cells were heated at 44 ℃ (Period-A), cultured at 37 ℃ as an interval (period-B) and heated again at 44 ℃ (period-C).
(b) step-up heating: Cells were treated at 42 ℃ for low heating (period-A) and followed by treatment at 44 ℃ as high heating (period-B).

or low heating, was performed at 42℃ for varied time from 0 to 2 houes (period-A), and followed by the second heating, or high heating, at 44 ℃ for the assay of the surviving fraction (period-B). The surviving fraction was determined with colony formed by culturing cells in 0.3% soft agar.

Drugs used were 10ug/ml of cepharanthin (Kaken Seiyaku Co., Tokyo) and 5ug/ml of cisplatin (Nippon Kayaku Co., Tokyo). All experiment were repeated at least three times and data shown in Figures are averages from these experiments.

Results

Fig. 2 shows survival curves of FM3A cells heated at temperatures from 41 to 46 ℃ . The surviving fraction decreased with elevation of the temperature. Fig. 3 shows survival curves when cells were heated with the presence of drugs. Cepharanthin (CPR) showed a small sensitizing effect (Fig. 3-a), and cisplatin (CDDP)showed a marked cell killing at 37 ℃ and sensitizing effect in elevated temperatures (Fig. 3-b). The sensitizing effects were compared using T_0 values defined by the time reducing the surviving fraction to 37% on the exponentially decreasing part of tne survival curves (Table 1).

Survival curves of cells for fractionated heating (FH) were shown in Fig. 4. The first heating was at 44 ℃ for 15 minutes and the period of the interval at 37 ℃ were 0, 2, 4

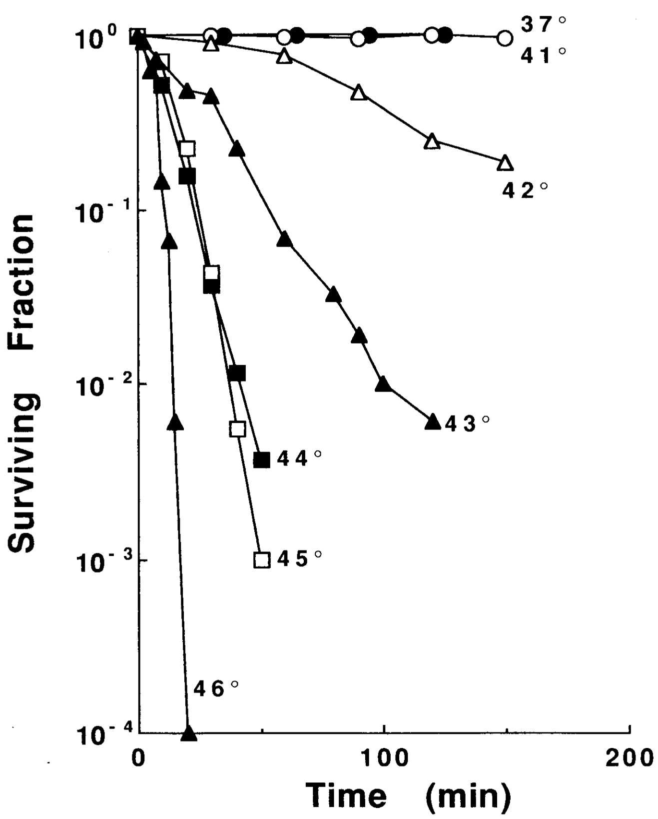


Fig. 2. Survival curves of FM3A cells treated at various temperatures from 41 to 46℃.

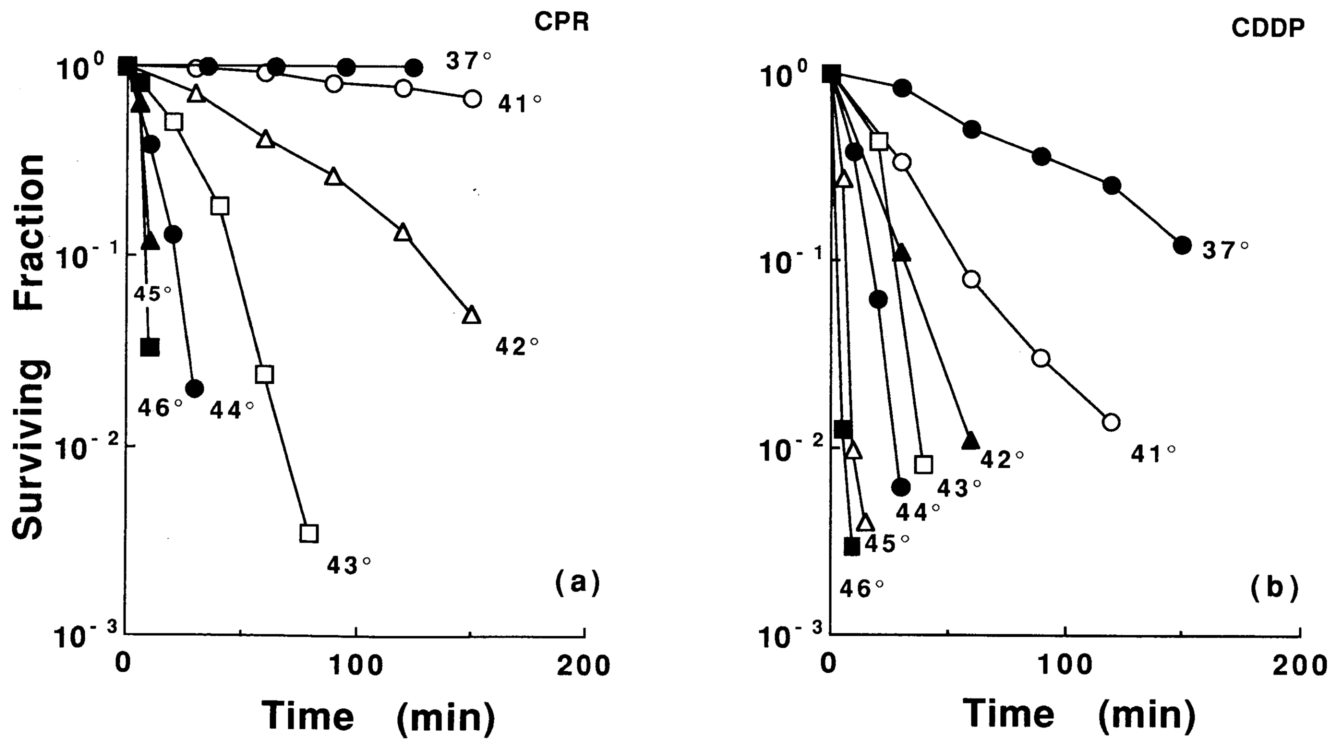


Fig. 3. Survival curves of cells treated at various temperatures with (a) cepharanthin, CPR, and (b) cisplatin, CDDP.

Table 1. T_0 values for different temperatures.

	37℃	41℃	42℃	43℃	44℃	45℃	46℃
Control	14,122	3,790	61.7	21.7	7.96	2.72	1.31
CPR	494	396	55.1	11.8	6.73	2.99	1.56
CDDP	66.2	27.5	13.3	5.05	4.84	2.51	1.72

Unit: minutes

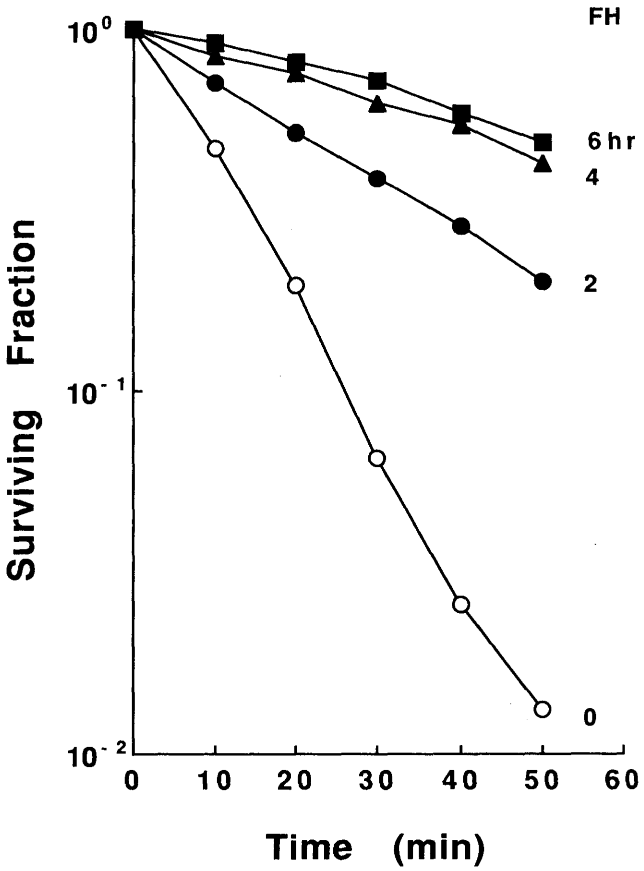


Fig. 4. Induction of thermotolerance by fractionated heating, FH. The first heating was at 44 ℃ for 15 minutes, and the intervals were varied from 0 to 6 hours. Survival curves were obtained at the second heating at 44℃.

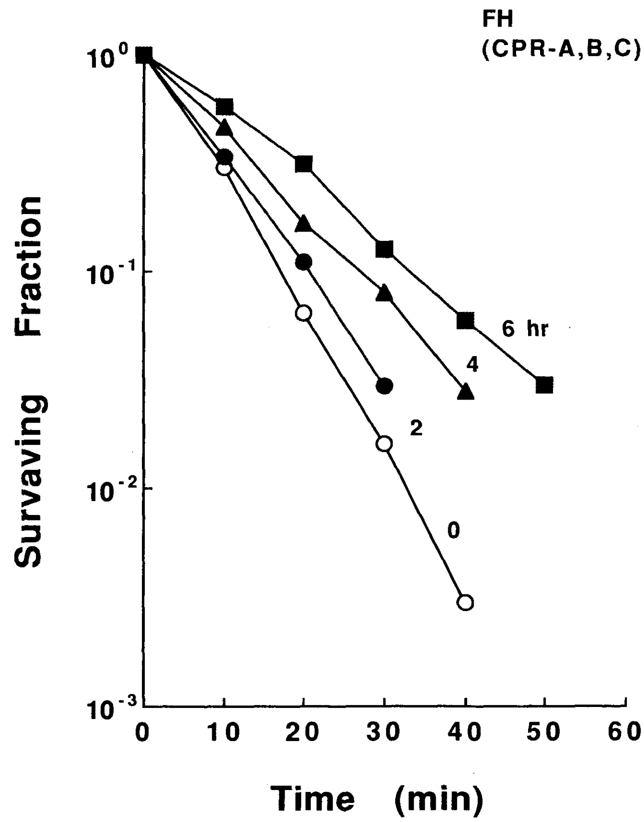


Fig. 5. Suppression by cepharanthin, CPR, of thermotolerance induced by fractionated heating. Cepharanthin was added during the period-A, B, and C.

and 6 hours. The increase in the surviving fracion with the period of the interval means the induction of thermotolerance. When cepharanthin (CPR) was added at the first heating and presented until the second heating, or the period-A, B and C, the surviving fraction decreased, indicating the suppression of thermotolerance (Fig. 5). The effects of cepharanthin added at different periods are shown in Fig. 6 for the period-A and the period-B, and in Fig. 7 for the period-C and the period-B and C. T_0 values obtained from Figs. 6 and 7 are shown in Table 2. The suppression of thermotolerance was maximum when cepharanthin was present from the beginning to the end of the treatment, or the period-A, B and C. The thermotolerance ratios, TTR, under the conditions with or without cepharanthin were compared in Fig. 8. TTR is the ratio of a T_0 value for the period of the interval indicated in Fig. 8 to that for the interval 0 hour. TTR increased with the period of the interval in the fractionated heating. When cepharanthin was added at the period-A, there was no effect. For the period-B and the period-C, thermotolerance was suppressed by the same extent. When added during the period-B and C, the suppression were enhanced. When cepharanthin was further presented during the period-A in addition to the period-B and C, or the period-A, B and C, the suppression became the maximum. TTR when cepharanthin was added during the period-A, B and C was reduced to 0.22 of the control value at the interval of 6 hours.

The effect of cisplatin (CDDP) was examined for the induction of thermotolerance in fractionated heating. As cisplatin was more toxic than cepharanthin, it was added only at the period-C. The surviving fraction at different

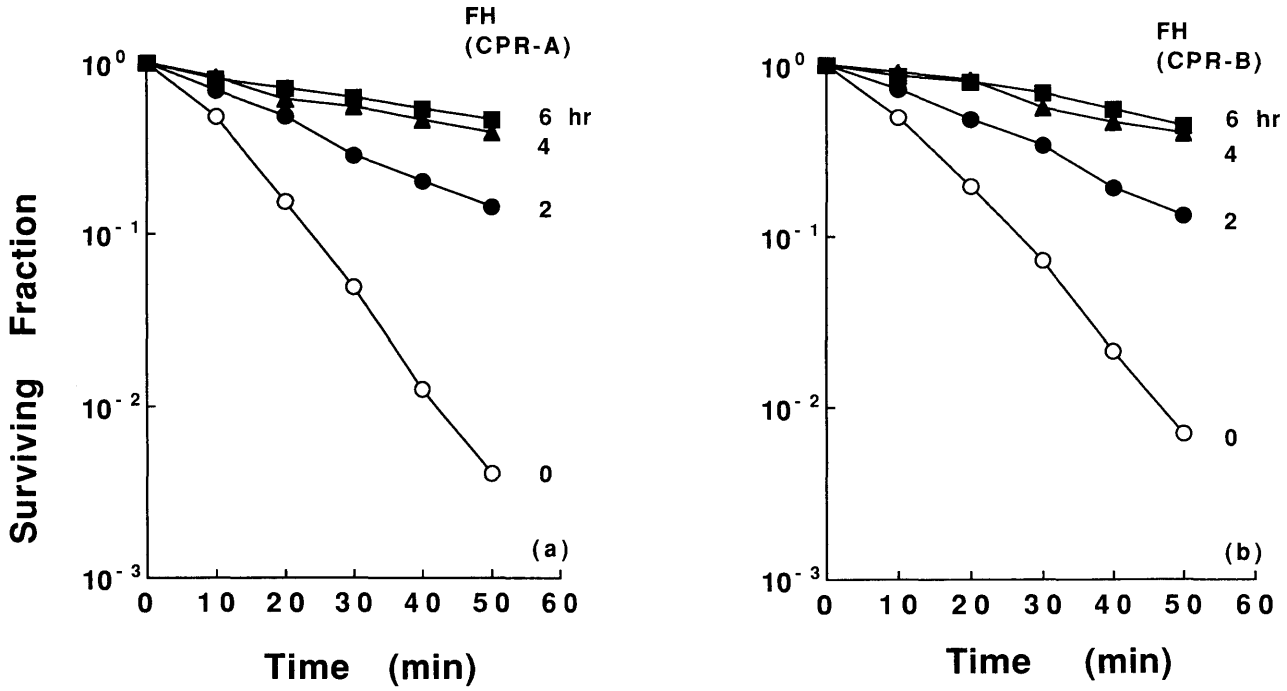


Fig. 6. Supperssion by cepharanthin, CPR, of thermotolerance induced by fractionated heating. Cepharanthin was added during (a) the period-A and (b) the period-B.

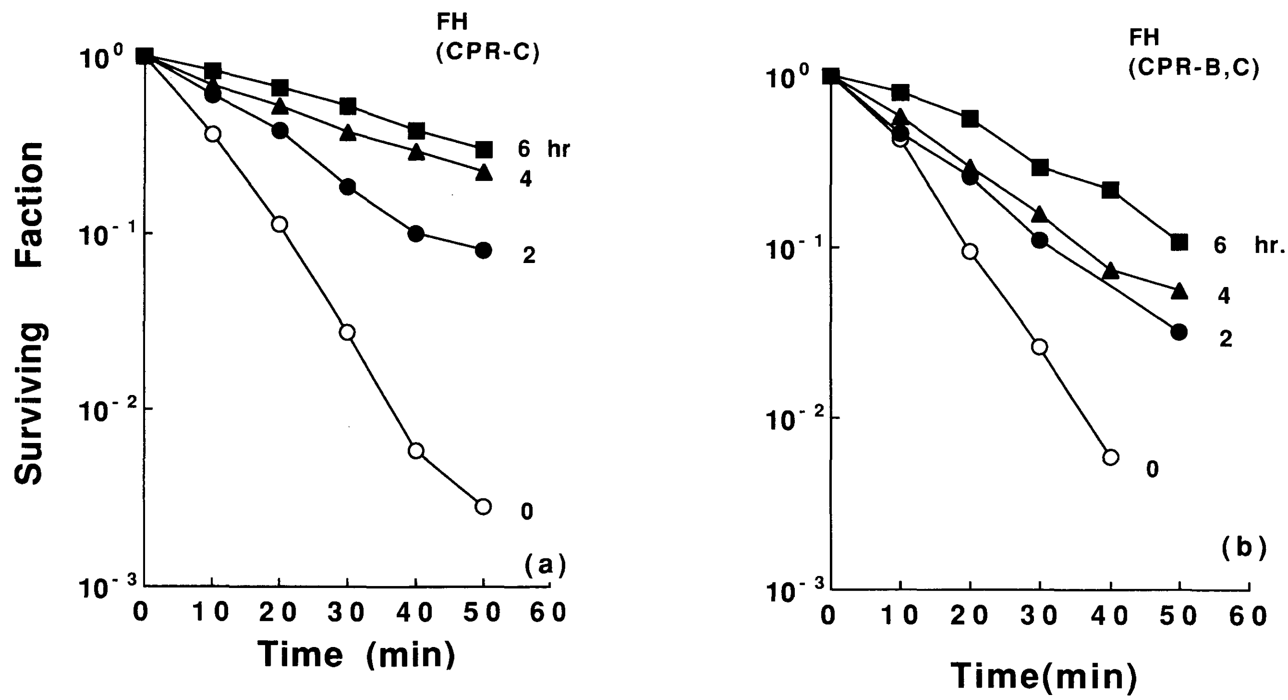


Fig. 7. Suppression by cepharanthin, CPR, of thermotolerance induced by fractionated heating. Cepharanthin was added during (a) the period-C and (b) the period-B and C.

Table 2. T_0 values for different periods of interval in fractionated heating.

	0 hr	2 hr	4 hr	6 hr
Control	10.2	31.6	65.6	90.7
CPR-A, B, C	6.58	8.60	10.9	12.9
-A	8.74	24.7	56.3	71.3
-B	8.91	23.0	42.1	49.3
-C	7.28	18.5	32.7	38.4
-B, C	7.10	15.4	14.8	20.1
CDDP-C	8.14	17.6	24.0	24.3

Unit: minutes

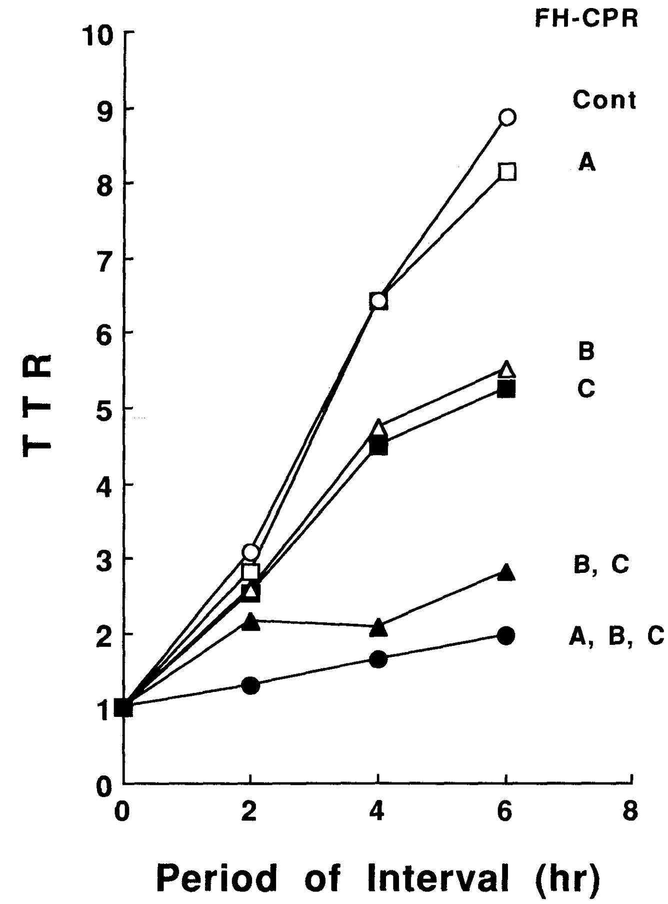


Fig. 8. Change in thermotolerance ratio, TTR. with the period of the interval at 37 ℃ , and suppression of thermotolerance by addition of cepharanthin at different periods.

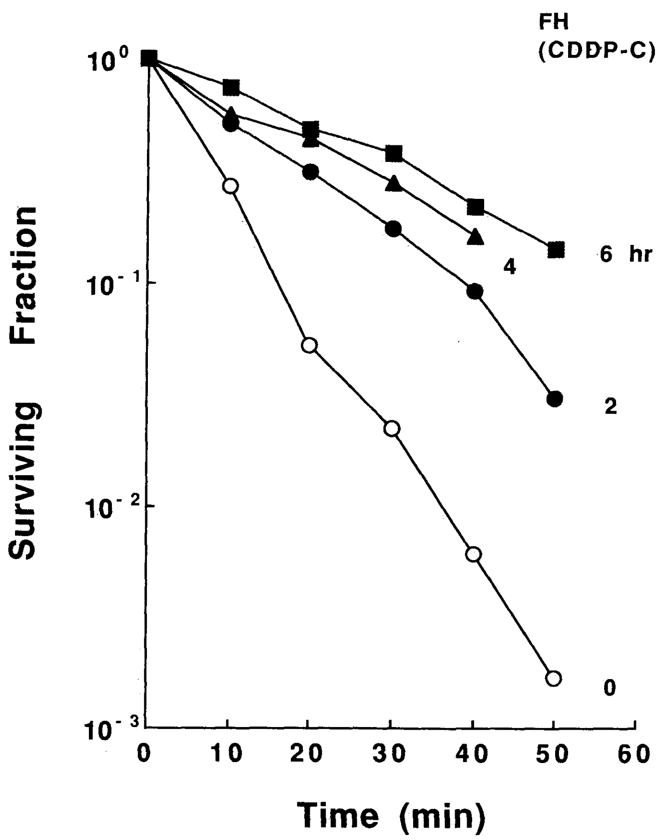


Fig. 9. Suppression by cisplatin, CDDP, of thermotolerance induced by fractionated heating. Cisplatin was added during the period-C.

Table 3. T_0 values for different periods of low heating in step-up heating.

	0 hr	1 hr	1.5 hr	2 hr
Control	7.96	12.1	26.7	39.6
CPR-A, B	6.95	10.2	12.6	14.8
-B	6.95	9.50	13.4	15.1
CDDP-A, B	5.69	8.75	9.71	10.5
-B	6.54	9.53	10.5	12.4

Unit: minutes

intervals was shown in Fig. 9, and T_0 values were shown in Table 2. TTR calculated from Table 3 is shown in Fig. 10. Cisplatin also suppressed thermotolerance.

Kinetics of thermotolerance induced by step-up heating (SUH) was examined. Survival curves of cells treated with step-up heating with periods of the low heating for 0, 1, 1.5 and 2 hours were shown in Fig. 11. Thermotolerance was induced with the period of the low heating. Survival curves when cepharanthin was added at the period-A and B, and the period-B were shown in Fig. 12. The addition of cepharanthin reduced the surviving fraction, indicating that suppression of thermotolerance by cepharanthin. Their T_0 values were shown in Table 3. TTR was calculated as a ratio of a T_0 value for the corresponding period of the low heating to that for the period 0 hour, and is shown in Fig. 13. TTR of control increased slowly until 1 hour of the low heating, and then increased rapidly. The suppression of thermotolerance was marked after 1.5 hours of the low heating with the presence of cepharanthin. There was no difference in the suppression when cepharanthin was added at the period-A and B or the period-B, indicating that cepharanthin was effective when added at the period-B but not effective at the period-A. TTR was reduced to 0.44 of the control value when cepharanthin was added for 2 hours of low heating.

The effect of cisplatin on the suppression of thermotol-

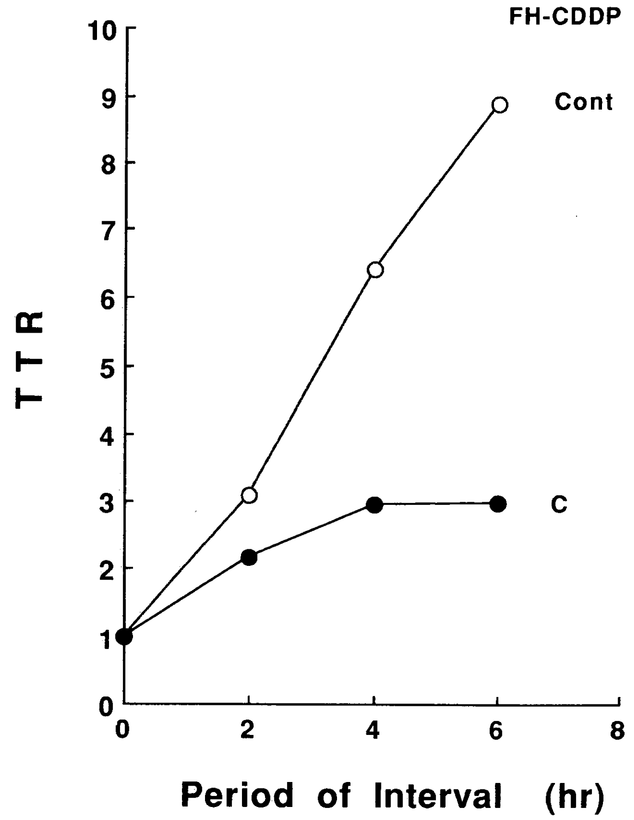


Fig. 10. Suppression of thermotolerance ratio, TTR, in fractionated heating by addition of cisplatin at the period-C.

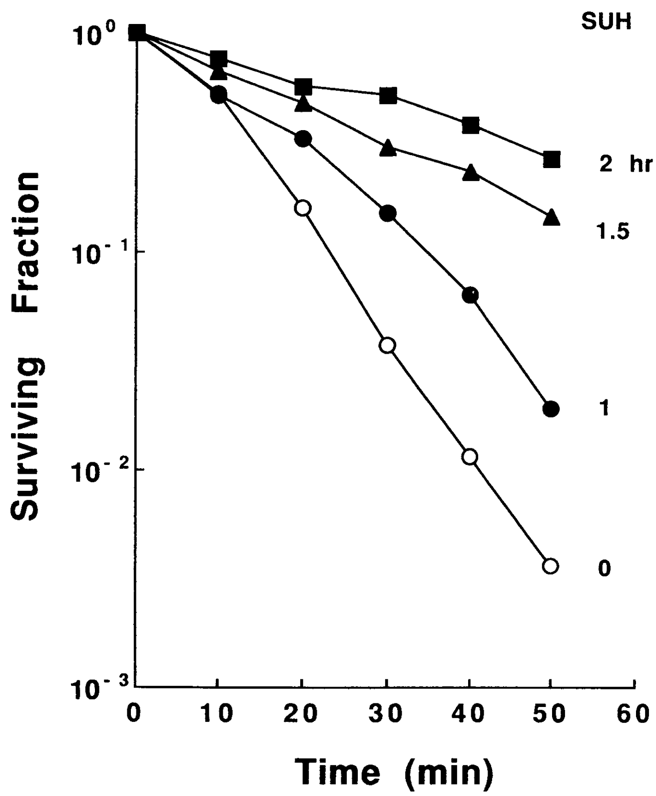
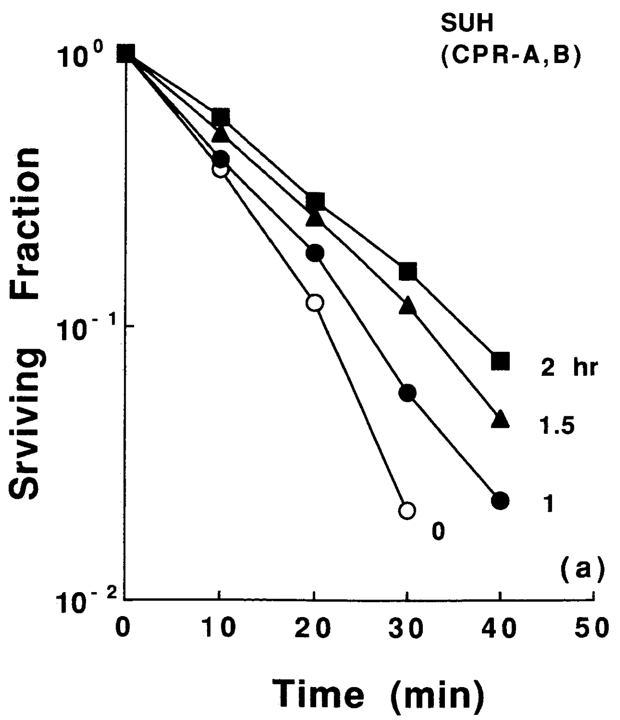


Fig. 11. Induction of thermotolerance by step-up heating, SUH. The low heating was at 42℃ for various period from 0 to 2 hours. The survival curves were obtained at the high heating at 44℃.



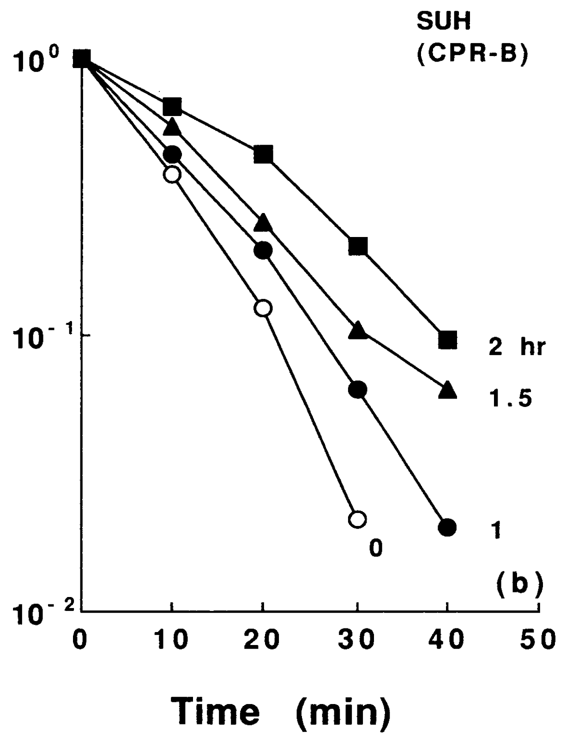


Fig. 12. Suppression by cepharanthin, CPR, of thermotolerance induced by step-up heating. Cepharanthin was added during (a) the period-A and B and (b) the period-B.

erance was examined in the step-up heating. Fig. 14 shows survival curves when cisplatin was added at the period-A and B and the period-B. The alteration of TTR by the addition of cisplatin was shown in Fig. 15. The suppression of thermotolerance by cisplatin was effective at the period-B but not at the period-A as the similar way to cepharanthin (Fig. 13).

Discussion

By elevating temperature of cancer cells above 42 ℃ , cells receive lethal damages and eventually will die. The lethal effect becomes significant with elevation of the temperatures. This thermal killing of cells is applied for cancer treatment as hyperthermia. In the application of hyperthermia to clinical treatments, following points should be established: (1) Instruments for heating cancer tissues selectively at a planned temperature higher than 42 ℃ . (2) Methods noninvasively measuring temperatures of the cancer tissues during treatments. (3) Biological modification to thermosensitize cancer cells. Many studies have been performed for the establishment of above points,[1] but further efforts should be taken.

In the induction of thermotolerance three processes would exist: heat stress, development and expression. The heat stress might be occurred at temperatures above 42 ℃ . The development might proceed at 37℃ , and also at 42℃ . The expression is observed at the time to assay the thermal cell killing. In this study, the temperatures for the heat

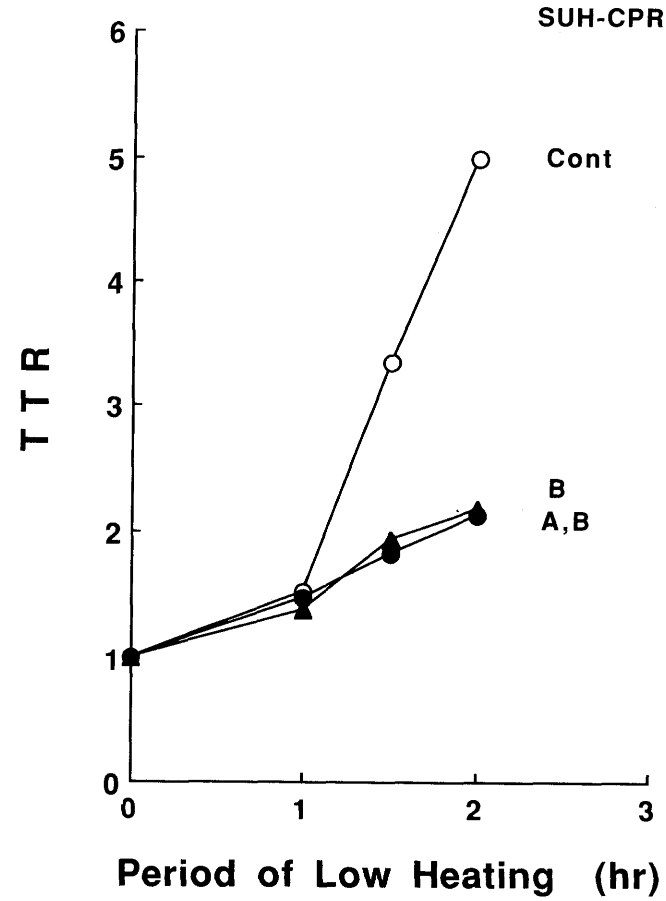


Fig. 13. Suppression of thermotolerance Ratio, TTR, in step-up heating by addition of cepharanthin.

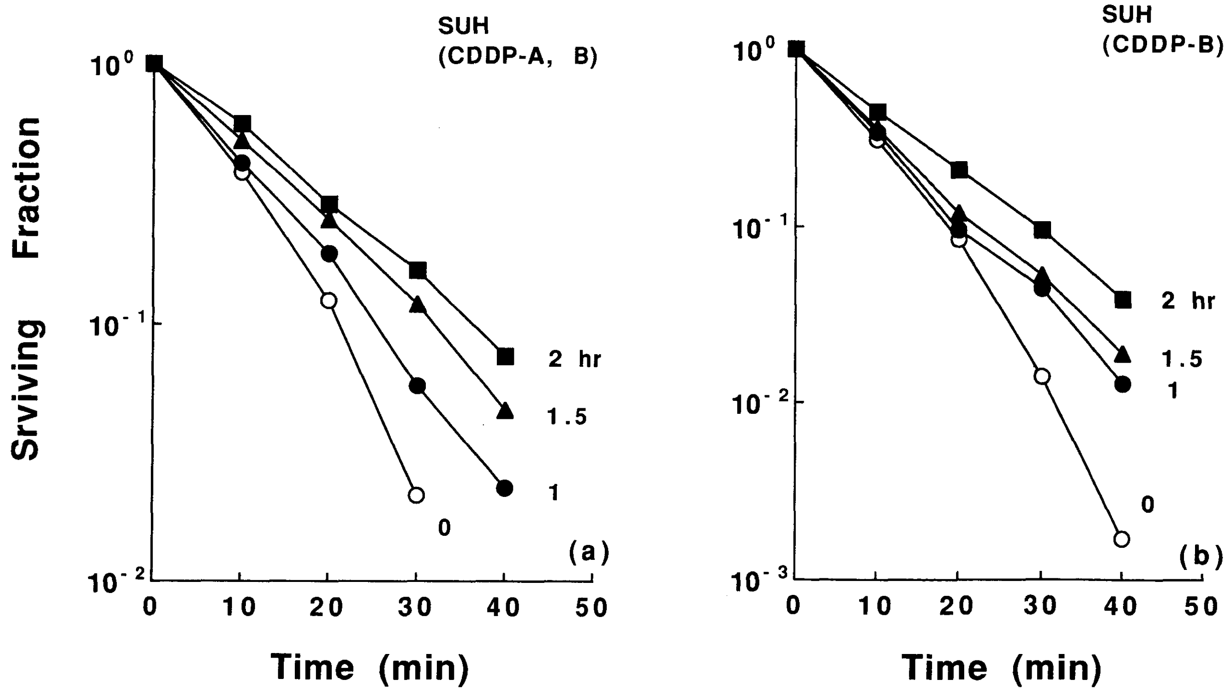


Fig. 14. Suppression by cisplatin, CDDP, of thermotolerance induced by step-up heating. Cisplatin. was added during (a) the period-A and B and (b) the period-B.

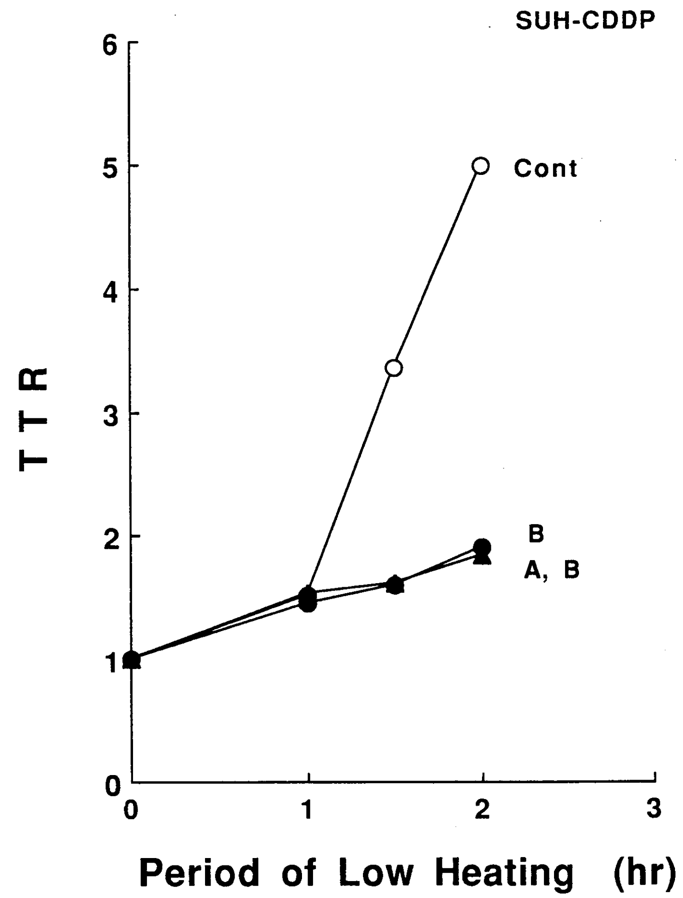


Fig. 15. Suppression of thermotolerance Ratio, TTR, in step-up heating by addition of cisplatin.

stress, the development and the expression were 44, 37 and 44 ℃ for the fractionated heating, respectively, and 42, 42 and 44 ℃ for the step-up heating, respectively. The amount of induced thermotolerance was increased with the periods of the heat stress and the development (Figs. 4 and 11).

Cepharanthin was used for the suppression of thermotolerance. Cepharanthin is an alkaloid acting as a modifier of cell membrane and protect from hemolysis by snake venom.[37,38] Cepharanthin has small toxicity at 37 ℃ (Fig. 3-a), but suppressed thermotolerance in fractionated heating when added at the steps of the development and the expression (the period-B and the period-C, Fig. 8), but not in the process of the heat stress (the period-A, Fig. 8). In step-up heating cepharanthin suppressed thermotolerance at the step of the expression, not at the step of heat stress and the development (Fig. 13). As cepharanthin is toxic at 37 ℃ , it can be effectively applied to hyperthermia for the suppression of thermotolerance.

Cisplatin was also used to examine the suppression of thermotolerance. Cisplatin has been used clinically as an anticancer drug and also can be used in combination with hyperthermia.[12-16] As cisplatin is highly toxic to cells, it was added only at the period-C in fractionated heating which is the process of the expression, and showed marked suppression of thermotolerance (Fig. 10). In step-up heating cisplatin acted as cepharanthin did (Fig. 13). As cisplatin has been used in chemotherapy, it also can be combined with hyperthermia for the suppression of thermotolerance.

The induction of thermotolerance is associated with the hest shock proteins.[39-44] It has been reported that several heat shock proteins were observed during the induction of thermotolerance. However, the mechanism of the induction of thermotolerance is not well analyzed in relation to the heat shock proteins. The mechanism of the suppression of thermotolerance by cepharanthin and cisplatin is not well explained and needs further study, though some relationship might exist between the suppression of thermotolerance and heat shock protein (s).

Acknowledgment

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