

Benzylidene Lactam Compound, KNK437, a Novel Inhibitor of Acquisition of Thermotolerance and Heat Shock Protein Induction in Human Colon Carcinoma Cells

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ABSTRACT

Cells exposed to heat or other types of stressors transiently synthesize a group of proteins known as heat shock proteins (HSPs). A nonlethal heat treatment can elicit in the cells an ability to resist subsequent lethal heat treatments. We report here that a novel benzylidene lactam compound, KNK437, dose-dependently inhibited the acquisition of thermotolerance and the induction of various HSPs including HSP105, HSP70, and HSP40 in COLO 320DM (human colon carcinoma) cells. The induction of heat-inducible HSP70, which is reported to be involved in the development of thermotolerance, was inhibited at mRNA levels by treatment with KNK437. This compound also inhibited the acquisition of thermotolerance as developed by sodium arsenite. However, it did not increase thermosensitivity in nontolerant cells. The effect of KNK437 was much greater than that of quercetin, a bioflavonoid that was previously reported to inhibit the acquisition of thermotolerance as well as the induction of HSPs. We conclude that this drug is a novel inhibitor of the acquisition of thermotolerance caused by the induction of HSPs.

INTRODUCTION

Hyperthermia is an effective modality for cancer therapeutics, particularly in conjunction with radiotherapy, to obtain greater therapeutic benefits. One of the major problems of hyperthermia is the development of thermotolerance, a transient resistance to heat induced by prior sublethal heat treatment (1, 2). The inhibition of the acquisition of thermotolerance could be expected to improve the antitumor efficiency of hyperthermia. Although the mechanisms of thermotolerance have not been definitively elucidated, HSPs² are involved in the acquisition of thermotolerance in mammalian cells to protect them from subsequent damage (3, 4).

HSPs are a unique group of proteins induced by heat or other stressors such as sodium arsenite, heavy metals, or amino acid analogues in prokaryotic and eukaryotic cells. In mammalian cells, the expression of a number of HSPs, including HSP40, HSP47, HSP70, HSP90, and HSP100, is enhanced by heat shock and regulated at the transcriptional level (5). HSPs bind to denatured proteins caused by various stresses including heat shock and serve to renature those proteins or to bring them to the degradation pathway. Some of the HSPs also have important roles as molecular chaperones for the maintenance of the intracellular environment, regulating the protein folding and translocation of proteins into endoplasmic reticulum, mitochondria, and so on (6).

On the other hand, the induction of HSPs by hyperthermic treatment contributes to the acquisition of thermotolerance in tumor cells. Various studies have shown a close relationship between the level of thermotolerance and the cellular content of HSP72, the inducible form

of HSP70 family proteins. For example, microinjection of antibodies against HSP70 makes cells more sensitive to thermal stress (7), and HSP72 is associated with ribosomal subunits in thermotolerant cells, but not in normal cells (8). HSP synthesis induced by heat shock is mainly regulated by HSF1 and HSE in the promoter region (9–11). HSF1 exists in unstressed cells in an inactive form and is rapidly activated after heat shock.

The effectiveness of hyperthermia will definitely improve if the induction of HSPs including HSP70 in tumor cells is inhibited during heat treatment. For that reason, it is desirable to inhibit activation of HSF1 during heating for specific inhibition of inducible HSPs without affecting the expression and function of constitutive forms of HSPs. We reported previously that the bioflavonoid quercetin inhibited the synthesis of various HSPs, including inducible HSP70, as induced by heat shock or treatment with sodium arsenite in COLO 320DM human colon carcinoma cell lines (12, 13). Quercetin inhibited the induction of HSP70 at the mRNA level through inhibition of HSF1 activation (13) or was reported to cause retardation of the induction of HSP70 mRNA (14). Quercetin was also reported to inhibit the acquisition of thermotolerance in COLO 320DM cells (15). Thus far, quercetin is the only compound that has been proven to be involved in the inhibition of induction of HSPs as well as in the acquisition of thermotolerance.

In this study, we found a benzylidene lactam compound, KNK437, to be an inhibitor of the acquisition of thermotolerance. It was more effective than quercetin in inhibiting acquired thermotolerance and in inhibiting various HSPs at the mRNA level. These results demonstrate the usefulness of this compound as an effective sensitizer for cancer hyperthermic therapy and also suggest the strong relationship between thermotolerance and HSPs.

MATERIALS AND METHODS

Cells and Culture Conditions. COLO 320DM cells (16) derived from a human colon cancer and HeLa S3 cells were provided by the American Type Culture Collection. Murine transplantable carcinoma SCC VII cells were kindly provided by Dr. M. Hiraoka (Kyoto University, Kyoto, Japan). Cells were cultured in DMEM supplemented with 10% fetal bovine serum in a humidified 5% CO₂ atmosphere at 37°C.

Hyperthermia and Drug Treatments. Heat treatments at 42°C for 90 min were performed in a CO₂ incubator using 25-cm² plastic flasks. Cells (1 × 10⁵) were seeded in the flasks, incubated at 37°C for 48 h, and then heated by immersing the flasks in a water bath (45°C ± 0.05°C). KNK437 (*N*-formyl-3,4-methylenedioxy-benzylidene- γ -butyrolactam; synthesized by Kaneka Corp., Osaka, Japan) and quercetin (Nacalai Tesque, Kyoto, Japan) were dissolved in DMSO before being added at the indicated concentrations. The final concentration of DMSO in each culture medium was 0.25% (v/v), irrespective of the concentrations of the drugs. The same concentration of DMSO was used as a control. Sodium arsenite was dissolved in PBS at a concentration of 80 mM and added to the medium. Cells were treated with 300 μ M sodium arsenite at 37°C for 1.5 h, rinsed, and then incubated at 37°C for 5 h before 45°C heat challenge.

Colony-forming Assay. Cells were trypsinized, counted, and replated at the appropriate dilutions. Surviving colonies were counted after 10–12 days of growth at 37°C. Plating efficiencies were routinely about 80–90%. A set of at

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² The abbreviations used are: HSP, heat shock protein; HSF, heat shock factor; HSE, heat shock element.

least six dishes was used for each experimental condition. The surviving fraction was calculated as the plating efficiency of the treated cells divided by the plating efficiency of untreated control cells.

Metabolic Labeling and Gel Electrophoresis. COLO 320DM cells (2×10^5) were injected into each well of 12-well plastic plates 2 days before incubation in the presence of KNK437 for 1 h before heat shock. The cells were then heat-shocked at 42°C for 90 min or kept at 37°C for the same length of time and incubated at 37°C for 2 h. KNK437 or 0.25% DMSO, was present in the medium from 1 h before heat shock until the end of the 2-h recovery period. For metabolic labeling, cells were washed with PBS without Ca^{2+} or Mg^{2+} and incubated for 1 h with 1.22 MBq of [^{35}S]methionine in 250 μl of methionine-free DMEM supplemented with 10% dialyzed fetal bovine serum. After metabolic labeling, cells were washed twice with PBS and lysed in a buffer containing 1% NP40, 0.15 M NaCl, 50 mM Tris-HCl (pH 8.0), 5 mM EDTA, and protease inhibitors [0.2 mM 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride, 2 mM *N*-ethylmaleimide, 1 $\mu\text{g}/\text{ml}$ pepstatin, and 1 $\mu\text{g}/\text{ml}$ leupeptin]. After centrifugation at $12,000 \times g$ for 20 min, cell extracts containing equal amounts of trichloroacetic acid-insoluble radioactivity were analyzed by two-dimensional gel electrophoresis [the one-dimensional gel electrophoresis was a nonequilibrium pH gradient gel electrophoresis, and the two-dimensional gel electrophoresis was 10% SDS-PAGE (17, 18)].

Immunoblot Analysis. About 4×10^5 cells were injected in each well of 6-well plastic plates 2 days before a 1-h incubation with or without KNK437 before heat shock. The cells were heat-shocked and recovered as described above without labeling with [^{35}S]methionine. After washing, the cells were lysed in Laemmli's SDS sample buffer (18) and sonicated. Cell extracts containing equal amounts of protein were separated by one-dimensional SDS-PAGE according to the methods of Laemmli (18) and blotted on nylon membrane (19). After transfer, the membrane was incubated with monoclonal anti-HSP72 antibody (SPA-810; Stressgen Biotechnologies Co., Victoria, British Columbia, Canada) or anti- β -actin antibody (MAB1501; Chemicon, Temecula, CA) and then incubated with peroxidase-conjugated goat antibody against mouse IgG (Cappel; Organo Teknika Co., West Chester, PA), followed by detection with enhanced chemiluminescence Western blotting detection reagents (Amersham, Little Chalfont, Buckinghamshire, England).

Northern Blot Analysis. Cells (2×10^6) were plated in 25-cm² plastic flasks and treated on the second day with or without KNK437 for 1 h. The cells were then heat-shocked at 42°C for 90 min or kept at 37°C. Cells were harvested immediately after heat shock, and total RNA was purified, with minor modifications, according to methods described previously (13). Equal amounts of total RNA were electrophoresed in formaldehyde-containing agarose gels, transferred to a nylon membrane, and hybridized with [α - ^{32}P]dCTP-labeled probes for HSP70 (pH 2.3 H-B; 2.3 kb; kindly provided by Dr. R. I. Morimoto, Northwestern University, Chicago, IL) or β -actin as an internal control.

RESULTS

Inhibitory Effect of a KNK437 on the Acquisition of Thermotolerance. The effect of a KNK437, a benzylidene lactam compound (Fig. 1), on the acquisition of thermotolerance was examined in COLO 320DM cells by a protocol of fractionated heat treatment. As shown in Fig. 2, the thermotolerance was induced in COLO 320DM cells after the first heat treatment at 45°C for 10 min in the presence of DMSO (control). However, the acquisition of thermotolerance was almost completely inhibited by the presence of 100 μM KNK437 when this compound was added to the culture medium 1 h before the first heat treatment and remained present until the end of the second heat treatment (Fig. 2).

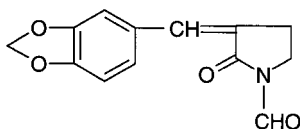


Fig. 1. The structure of KNK437 (*N*-formyl-3,4-methylenedioxy-benzylidene- γ -butyrolactam).

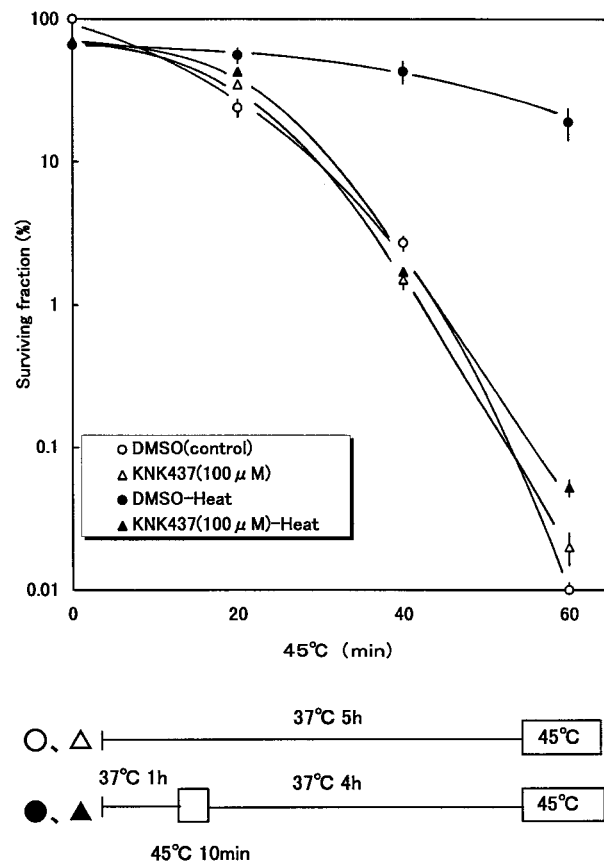


Fig. 2. Inhibition of thermotolerance by KNK437. Cells were preincubated at 37°C with (Δ) or without (\circ) KNK437 for 5 h before a challenging heat shock at 45°C. Cells preincubated with (\blacktriangle) or without (\bullet) KNK437 for 1 h before the first heat treatment at 45°C for 10 min were recovered at 37°C for 4 h and then treated with a challenging heat shock at 45°C for the indicated periods. Surviving fractions of the treated cells were examined as described in "Materials and Methods," and the results are expressed as the means of triplicate samples. Bars, SD.

To evaluate the dose-dependent effect of the drug on thermotolerance, cells were heated at 42°C for 90 min, incubated at 37°C for 2 h, and then treated at 45°C for the indicated periods. KNK437, which was present during the whole period of fractionated heat treatment, inhibited the acquisition of thermotolerance in a dose-dependent manner (Fig. 3A). A similar dose-dependent inhibitory effect of KNK437 on the acquisition of thermotolerance was also observed for a different cell line, HeLa S3 (Fig. 3B). The inhibitory effect was less prominent in HeLa S3 cells than in COLO 320DM cells, which may be attributed to the less effective inhibition of induction of HSPs by KNK437 in HeLa S3 cells (as shown later in Fig. 8). Here, we performed the preheat treatment at 42°C instead of 45°C, and these two different preheating conditions provided essentially the same effects on acquisition of thermotolerance (data not shown). KNK437 itself, even at a concentration of 200 μM , did not show any toxic effect on cell survival when cells were incubated at 37°C for 6 h (data not shown) or heated at 42°C for 90 min (Fig. 3A).

Quercetin was previously shown to have inhibitory effects on the acquisition of thermotolerance (15). Therefore, the inhibitory effects on acquisition of thermotolerance were then compared between these two compounds. As shown in Fig. 4, KNK437 inhibited the acquisition of thermotolerance much more effectively than quercetin when both compounds were examined at the same concentrations.

Sodium arsenite is an efficient inducer of the acquisition of thermotolerance as well as an inducer of HSPs in mammalian cells (15, 20). Treatment of COLO 320DM cells with 300 μM sodium arsenite

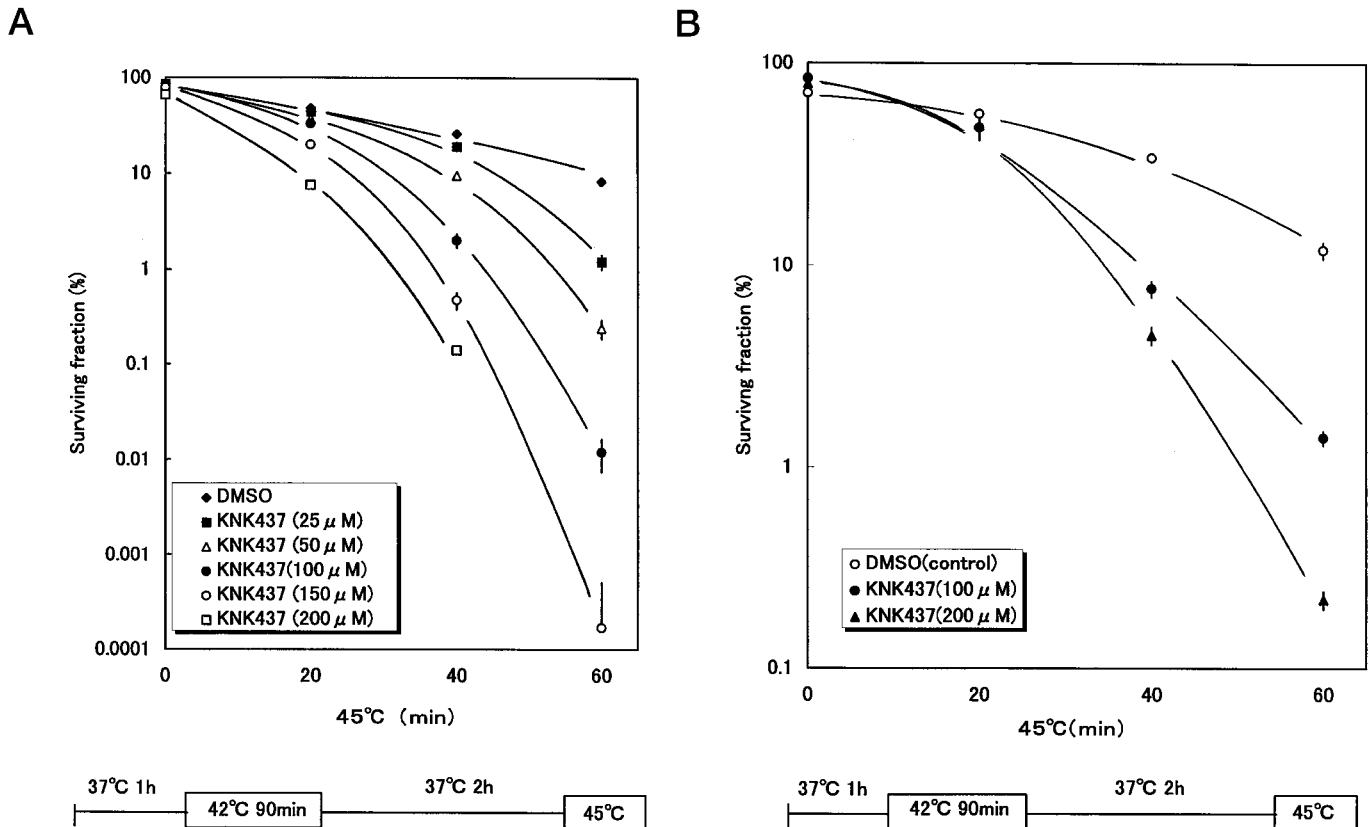


Fig. 3. Dose-dependent inhibition of thermotolerance by KNK437. A, COLO 320DM cells were incubated with KNK437 at concentrations of 0 (DMSO; ◆), 25 (■), 50 (△), 100 (●), 150 (○), and 200 μM (□) during the entire experimental period from 1 h before the first heat treatment. B, HeLa S3 cells were incubated with KNK437 at concentrations of 0 (DMSO; ○), 100 (●), and 200 μM (▲) during the entire experimental period from 1 h before the first heat treatment. Surviving fractions of treated cells were examined as described in "Materials and Methods", and the results are expressed as the means of triplicate samples. Bars, SD.

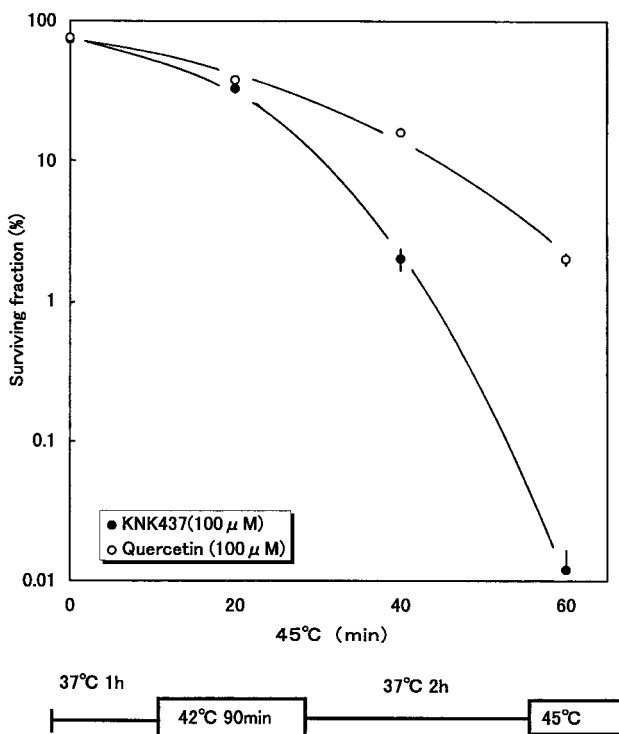


Fig. 4. Comparison of the inhibitory effects of KNK437 and quercetin on the acquisition of thermotolerance in COLO 320DM cells. Cells were incubated with 100 μM KNK437 (●) or quercetin (○) during the entire experimental period. Surviving fractions of treated cells were examined as described in "Materials and Methods," and the results are expressed as the means of triplicate samples. Bars, SD.

led to the development of thermotolerance in the cells (Fig. 5). The acquisition of thermotolerance induced by sodium arsenite was also inhibited by the presence of KNK437 (Fig. 5).

The inhibitory effect of KNK437 was then examined by changing the timing of its addition and the duration of the treatment. As shown in Fig. 6, cells were incubated with this compound: (a) for the first 3.5 h, including the first heat treatment period (group 1); (b) for the last hour and the second heat treatment period (group 2); or (c) for the entire first and second heat treatment periods (group 3). The inhibitory effect was most prominent when KNK437 was present during the entire period (group 3). When the compound was present only for the first 3.5 h (group 1), the inhibition was smaller than that seen in group 3. The inhibitory effect in group 2 was modest compared with that of the other groups, but it was still significant compared with the control (group 4). These data indicate that the presence of the compound during the first heat treatment is necessary for inhibition of the acquisition of thermotolerance. In light of these results, we next examined the effect of KNK437 on the induction of HSPs in COLO 320DM cells.

Inhibition of HSP Synthesis by KNK437. To examine the effect of KNK437 on the synthesis of HSPs, COLO 320DM cells were labeled with [^{35}S]methionine and analyzed by two-dimensional gel electrophoresis before and after heat shock. Major HSPs, such as HSP110 (21)/HSP105 (22, 23), HSP72, and HSP40 (24), were clearly induced after heat shock (Fig. 7). Adding 100 μM KNK437 to the medium during heat shock specifically inhibited the induction of these HSPs, whereas synthesis of other proteins remained essentially the same. At this concentration of KNK437, cell numbers neither increased nor decreased after 3 days of incubation. KNK437 did not

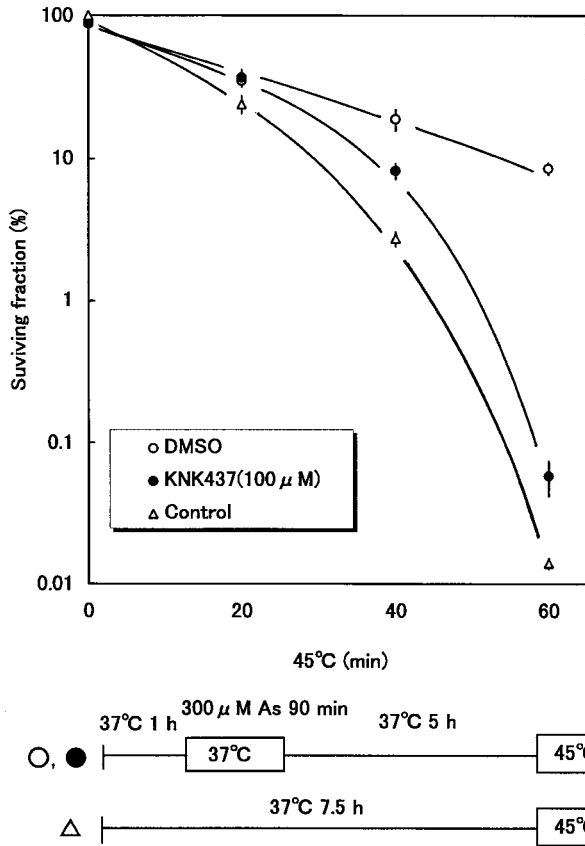


Fig. 5. Inhibition of thermotolerance induced by treatment with sodium arsenite. Thermotolerance was induced by incubating cells with 300 μM sodium arsenite for 90 min. Cells were preincubated with (●) or without (○) 100 μM KNK437 for 1 h before the sodium arsenite treatment. After treatment of the cells with sodium arsenite, cells were washed once with PBS and incubated at 37°C for 5 h with (●) or without (○) KNK437. As a control, the surviving fraction of the cells without KNK437 and sodium arsenite treatment during the entire experimental period (Δ) is also shown. The effects of KNK437 on acquired thermotolerance were tested by heating the cells at 45°C for the indicated time. Surviving fractions of the treated cells were examined as described in "Materials and Methods," and the results are expressed as the means of triplicate samples. Bars, SD.

inhibit the expression of constitutively expressed HSP family proteins such as HSC73 and HSP90 (Fig. 7, A and B, spots *d* and *e*).

Dose-dependent inhibition of the induction of HSP72 protein by KNK437 was examined using Western blot analysis (Fig. 8). KNK437 dose-dependently suppressed the level of HSP72 after heat shock in COLO 320DM cells (Fig. 8A), HeLa S3 cells, and SCC VII cells (Fig. 8B). To evaluate the inhibitory effect of KNK437 accurately, the amount of β-actin was also examined by Western blot analysis as an internal control. The inhibitory effect of KNK437 at the same concentration was higher for COLO 320DM cells than for HeLa S3 cells. This is consistent with the previous results that KNK437 inhibited the acquisition of thermotolerance less effectively for HeLa S3 cells than for COLO 320DM cells (Fig. 3, A and B). The inhibitory effect of KNK437 on the acquisition of thermotolerance was approximately 4-fold higher in COLO 320DM cells than in HeLa S3 cells (see Fig. 3, A and B), and the inhibitory effect on the induction of HSP72 was again about 4-fold higher for COLO 320DM cells than for HeLa S3 cells (compare Fig. 8A with Fig. 8B).

When the compound was removed from the culture medium after 24 h of incubation, cell proliferation started again, and the cell numbers recovered to the control level (data not shown), suggesting that the effect of KNK437 is cytostatic and reversible rather than cytotoxic. Next we examined whether the inducibility of HSPs is

restored when the compound is removed from the medium after preincubation with the cells. As shown in Fig. 8C, the induction of HSP72 by heat shock was clearly inhibited when COLO 320DM cells were preincubated with KNK437 for 24 h (Lane 2). However, when the COLO 320DM cells preincubated with KNK437 were further incubated for 24 h in medium without KNK437, the inducibility of HSP72 (Lane 4) was restored to the normal level without incubation with KNK437 (Lane 3).

To investigate the effect of KNK437 on the level of HSP70 mRNA, we performed Northern blot analysis (Fig. 9). HSP70 mRNA was markedly induced by heat treatment at 42°C for 90 min, and the accumulation of HSP70 mRNA by heat shock was almost completely inhibited by the presence of 100 μM KNK437. The level of β-actin mRNA as an internal control remained virtually unchanged under all of the conditions examined.

DISCUSSION

A number of reports have suggested a strong correlation between the induction of HSPs and the acquisition of thermotolerance in mammalian cells as well as yeast and bacteria (25, 26). Cells preheated at a sublethal temperature accumulate HSPs and are reported to acquire thermotolerance against a second heat challenge at a lethal temperature. Microinjection of anti-HSP70 antibody or transfection of

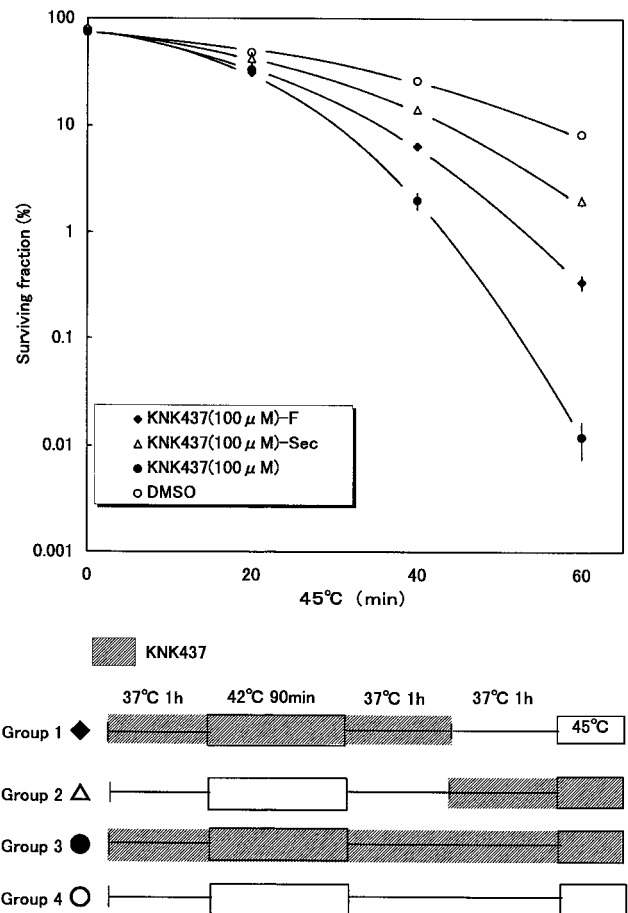


Fig. 6. Effects of KNK437 on the first heat treatment and the second lethal heat treatment. As shown in the bottom panel, cells were incubated with 100 μM KNK437 for the first 3.5 h, including the first heat treatment period (Group 1; ◆); for the last 1 h and the second heat treatment period (Group 2; △), and for the entire period including the first and second heat treatment periods (Group 3; ●). The surviving fraction of the thermotolerant cells without KNK437 treatment (○) is also shown. Results are expressed as the means of triplicate samples. Bars, SD.

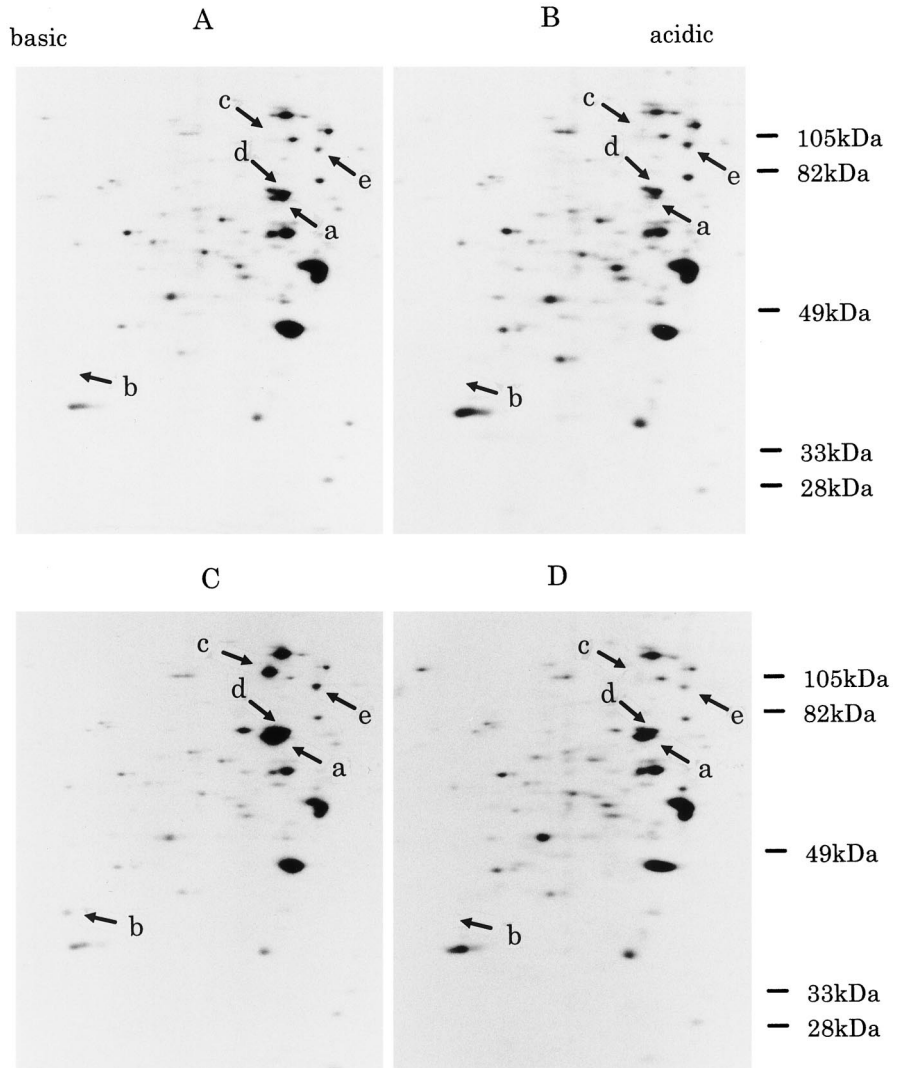


Fig. 7. Two-dimensional gel electrophoresis of COLO 320DM cells. Cells were treated with or without heat shock at 42°C for 90 min in the presence or absence of 100 μ M KNK437. After incubation at 37°C for 2 h, cells were labeled with 1.22 MBq of [³⁵S]methionine for 1 h. The compound was absent during the labeling period. A, vehicle (DMSO) without heat treatment. B, 100 μ M KNK437 without heat treatment. C, vehicle with heat treatment. D, 100 μ M KNK437 with heat treatment. Arrows, HSP72 (a), HSP40 (b), HSP105 (c), HSC73 (d), and HSP90 (e).

excessive amounts of cDNA encoding the HSP72 promoter region elevated thermosensitivity of the modified cells (7, 25). Cells stably transfected with cDNA encoding HSP70 showed thermoresistance for an otherwise lethal heat treatment.

Because it is critical for cancer therapies using hyperthermia to prevent the induction of HSPs, a specific inhibitor of induction of various HSPs in cancer cells after the first heat treatment would be useful for effective hyperthermic therapy. Quercetin, a bioflavonoid, was reported to inhibit the induction of HSPs including HSP70, HSP90, HSP47, and HSP27 at the mRNA levels (12, 13) and to prevent the acquisition of thermotolerance in COLO 320DM human colon cancer cells (15). Quercetin is the only specific inhibitor for the induction of HSPs reported thus far.

We report here that KNK437 dose-dependently inhibited the acquisition of thermotolerance after fractionated heat treatment. KNK437 is a novel compound that we first isolated from organic source libraries available in our laboratory. It does not show any anticancer effect by itself, nor does it inhibit the activities of protein kinases A and C and tyrosine kinase. A structurally similar compound shows antidepressant activity, but this pharmacological function has not yet been revealed.³

Inhibition of the acquisition of thermotolerance by this compound

was observed in COLO 320DM cells as well as in HeLa S3 cells (Fig. 3). The inhibitory effect was most prominent when this compound was added before the first heat treatment and was present throughout the experimental period, including the second heat treatment. This suggests that the prevention of thermotolerance acquisition in the cells treated with this compound may be due to the inhibition of HSP induction. As shown in Fig. 7, KNK437 inhibited the induction of various HSPs including HSP70, HSP40, and HSP105. HSP105 has a homologous region for HSP70 (21) and has been reported to contribute to the induction of thermotolerance in mouse cells (27). When KNK437 was added to the medium only during the second heat treatment at 45°C, it still showed a weak inhibitory effect, suggesting that the HSPs induced during the second heat treatment may also partially contribute to the induction of thermoresistance. The inhibitory effect on the acquisition of thermotolerance was also observed when the cells were pretreated with sodium arsenite. Thus, KNK437, as noted here, is the second reported specific inhibitor of the induction of thermotolerance as well as the induction of HSPs. This compound was more effective than quercetin in inhibiting the induction of thermotolerance, and the toxic effect of this compound on the cells was less than that of quercetin, suggesting that this compound might be a more useful potentiator for hyperthermic cancer therapy.

Although quercetin has been reported to inhibit the induction of HSP70 synthesis at the mRNA level (12), the inhibitory mechanism

³S. Yokota, K. Yamamoto, M. Kitahara, and K. Nagata, unpublished observations.

has not been clearly established. The binding of HSF1 to HSE is blocked when quercetin is added before and during heat treatment (13), and the phosphorylation of HSF1 is partially inhibited (28). This is consistent with a recent study (29) reporting that the targeted disruption of murine HSF1 abolishes thermotolerance as well as protection against heat-inducible apoptosis. On the other hand, quercetin is also reported as only delaying the synthesis of HSP70 after heat shock (14). Quercetin is also reported to have scavenger activity for reactive oxygen species, and reactive oxygen is reported to induce HSPs. Like quercetin, KNK437 also inhibits the induction of HSP70 at the mRNA level. Because KNK437 did not inhibit the constitutive expression of HSP family proteins as shown in Fig. 7, the inhibitory mechanism of this compound might be attributed to inhibition of the activation of HSF1 or the interaction of HSF1 with HSE as reported for quercetin (13). However, it should be more carefully studied, considering the involvement of other factors including reactive oxygen species (30, 31). KNK437 had an inhibitory effect on cell growth, which is reversible. However, there is no relationship between growth inhibition and inhibition of the acquisition of thermotolerance because we have already shown that cycloheximide inhibited cell growth but did not inhibit the acquisition of thermotolerance (15).

A number of studies (4, 32, 33) have demonstrated the possible

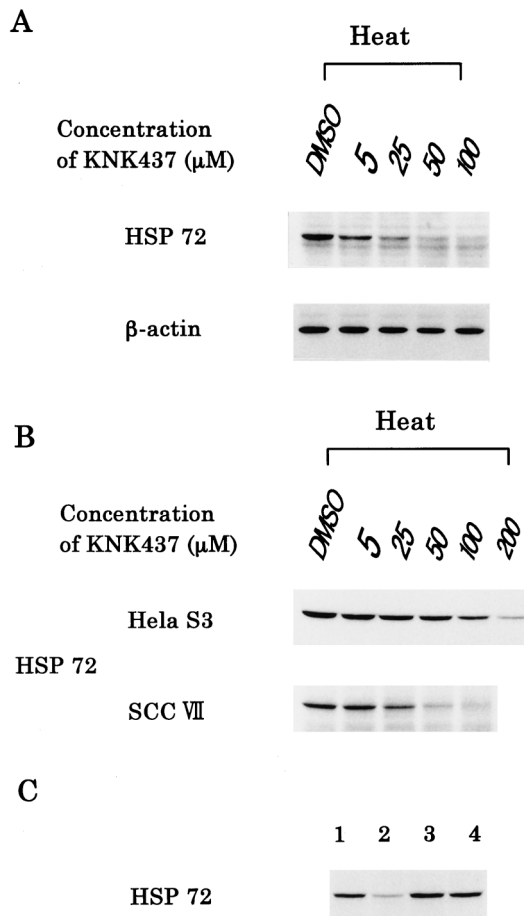


Fig. 8. Western blot analysis showing the effect of KNK437 on the induction of HSP72. COLO 320DM cells (A) or HeLa S3 and SCC VII cells (B) were pretreated with varying concentrations of KNK437 for 1 h and treated with heat shock at 42°C for 90 min. After incubation for 2 h at 37°C, cells were lysed and analyzed by Western blotting using the anti-HSP72 antibody. C, COLO 320DM cells were pretreated with (Lanes 2 and 4) or without (Lanes 1 and 3) 100 μM KNK437 for 24 h. The compound was removed from the culture medium, and cells were further incubated without KNK437 at 37°C for 1 h (Lane 2) or 24 h (Lane 4) and then treated with heat shock at 42°C for 90 min. After incubation for 2 h at 37°C, cells were lysed and analyzed by Western blotting using the anti-HSP72 antibody.

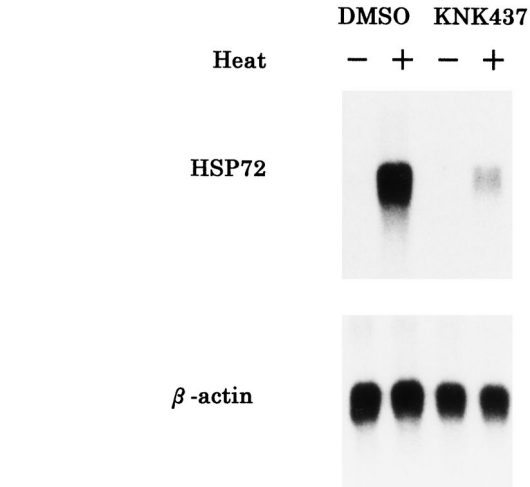


Fig. 9. Northern blot analysis of HSP72 mRNA. Cells were treated with or without heat shock at 42°C for 90 min in the presence or absence of 100 μM KNK437. Total RNA was extracted immediately after heat shock and analyzed using the cDNA encoding HSP72 as a probe.

involvement of apoptosis in killing tumor cells exposed to hyperthermia, and these studies suggest a strong relationship between the induction of stress proteins and the prevention of apoptosis. Quercetin was previously reported to induce apoptosis in tumor cells by inhibiting the induction of HSP70 synthesis after heat shock (34). Furthermore, inhibition of HSP70 synthesis as well as induction of apoptosis by treatment with quercetin combined with hyperthermia is reported to be confined to leukemic cells, and not to normal hematopoietic progenitor cells (35). The inhibition of Jun NH₂-terminal kinase activity by elevated levels of HSP70 is suggested to be responsible for the protection against apoptosis caused by various stresses including heat shock (36). The effect of KNK437 on the induction of apoptosis remains an area for future study.

In conclusion, we show here that KNK437 is a novel inhibitor for the acquisition of thermotolerance through inhibition of the induction of various HSPs. In addition, this compound has been found to enhance the efficiency of hyperthermic therapy against the growth of solid tumors transplanted into mice.⁴ We have also found that modified derivatives of KNK437 inhibited the induction of various HSPs after heat shock.³ Therefore, further screening of benzylidene lactam derivatives that inhibit the acquisition of thermotolerance more efficiently would contribute to the efficient application of hyperthermic therapy in human cancers.

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