Aspirin Enhances Thermotolerance in Human Erythroleukemic Cells: An Effect Associated with the Modulation of the Heat Shock Response

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ABSTRACT

Heat shock protein (HSP) synthesis is induced by hyperthermia and other types of stress in mammalian cells in vitro and in vivo. In the present report we describe that in human erythroleukemic cells, aspirin (400 μM), when administered during or immediately after a hyperthermic treatment, causes an increase in the amount of HSP70 synthesized and prolongs HSP70 synthesis for a period of several hours. This effect is not due to increased HSP70 mRNA stability. In the presence of aspirin, the heat shock transcription factor is maintained in the activated DNA-binding state for a period twice as long as control, an effect which results in enhanced and prolonged HSP70 mRNA transcription. A different cyclooxygenase inhibitor, indomethacin (10−5 M), also provokes similar effects. The modulation of the heat shock response by aspirin and indomethacin is associated with the ability of these drugs to potentiate the effect of hyperthermia and prolong thermotolerance for a period of 48 h. These results indicate that the use of aspirin and indomethacin should be carefully monitored in cancer patients undergoing hyperthermic treatment. On the other hand, the ability of aspirin to enhance HSP70 synthesis suggests that nonsteroidal anti-inflammatory drugs could potentiate the cytoprotective role of HSPs in pathological states, including fever, inflammation, and ischemia.

INTRODUCTION

Thermotolerance, the acquisition of a transient increased resistance to heat, is a universal noninheritable phenomenon, which can be induced by a short exposure to a nonlethal heat treatment. Mammalian cells expressing thermotolerance can exhibit survival levels several hundred times higher than normal cells when both are subjected to lethal hyperthermic temperatures (1, 2). For this reason, the development of thermotolerance in tumor cells is a major concern during hyperthermic treatment of cancer patients (2).

Although the molecular mechanisms responsible for the development of thermotolerance have not been elucidated, a fundamental role of HSPs3 is widely recognized (2–5). Like thermotolerance, the activation of a heat shock gene expression is a universal response of prokaryotic and eukaryotic cells to elevated temperatures or other environmental stresses (6, 7). HSPs enhance the ability of cells to recover from the toxic effects of heat and have an important role in cell protection during the pathogenesis of disease states, including hypoxia and inflammation (8). Their protective effect is mediated through several cellular activities, including binding to partially denatured proteins, dissociating protein aggregates, and regulating the correct folding and intracellular translocation of newly synthesized polypeptides (6–8). In mammalian cells, several HSPs are expressed during normal growth conditions and can be induced by biologically active molecules like hemin (9) and prostaglandins (10), whereas others are expressed upon stress-activated regulation of transcriptional and translational switches. Induction requires the activation and translocation to the nucleus of a transregulatory protein, the HSF from a monomeric non-DNA--binding form to an oligomeric form that binds to specific promoter elements (HSEs) located upstream of heat shock genes (7). Several HSFs have been identified in mammalian cells (7).

A role for arachidonic acid and its metabolites in the regulation of the heat shock response has been suggested recently by the evidence that arachidonic acid itself induces heat shock gene transcription and reduces the temperature threshold for HSF1 activation (11), whereas cyclopentenone prostaglandins, prostaglandin As and prostaglandin Js, induce HSP synthesis through cycloheximide-sensitive activation of HSF (10, 12). Prostaglandin A1 was also found to protect cells from thermal injury and to induce a thermostolerant state in human cells (13). It was interesting that the anti-inflammatory agent sodium salicylate, at concentrations as high as 20 mM, was also shown to induce HSF binding to the HSEs of the HSP70 gene, even though it was unable to induce HSP70 transcription (14).

In the present report, we investigated the effect of the anti-inflammatory drugs ASA and INDO, which inhibit the production of arachidonate metabolites via the enzyme COX (15, 16), on the development of thermotolerance and on the regulation of HSP70 gene expression in human K562 erythroleukemic cells. We demonstrate that ASA and INDO, at concentrations that do not inhibit cell proliferation, if administered during or immediately after a hyperthermic treatment, enhance thermotolerance in human erythroleukemic cells. This effect is associated with an enhancement of HSP70 gene transcription and with prolonged binding of HSF to HSE.

MATERIALS AND METHODS

Cell Culture and in Vitro Heating Procedure. K562 cells were maintained in RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, and antibiotics at 37°C in a 5% CO2 atmosphere. Cell numbers were counted in a hemocytometer, and cell viability was determined by dye exclusion technique, as reported previously (10). For heating procedure, flasks were submerged in a temperature-controlled water bath (Grant Instruments, Cambridge, England) at 45 ± 0.01°C (immersion depth = 4 cm, t1/2 = 1.5 min). A 20-min, 45°C treatment resulted in >80% cell survival. ASA, INDO (Sigma Chemical Co., St. Louis, MO), or ethanol diluent were added immediately after heat treatment and were kept during the recovery period at 37°C, unless differently specified. Statistical analyses were performed using a nonparametric test for unpaired data. Data are expressed as mean ± SD, and P values <0.05 were considered significant.

CSA. At different times after a primary HS and/or anti-inflammatory drug-treatment, cells were exposed to a lethal HS (45°C, 60 min), plated in 35-mm Petri dishes in culture medium containing 30% FCS and 0.3% Bacto Agar, and incubated at 37°C for 13–16 days. Only colonies of 100 or more cells were counted. Every sample was tested in duplicate, and every experiment was repeated at least 3 times. Survival values were adjusted for the plating efficiency of the appropriate control. Plating efficiencies were 60, 55, 46, or 44% for control, ASA-treated, INDO-treated, or HS-primed cells, respectively.

Protein Synthesis and Quantitative Immunoblot Analysis. K562 cells were labeled with [35S]methionine (10 μCi/2 × 106 cells) as described (10). After cell lysis, [35S]methionine-labeled proteins were separated by SDS-
PAG in a vertical slab gel apparatus (3% stacking gel, 10% resolving gel) and processed for autoradiography and densitometric analysis as described previously (10). For immunoblot analysis, equal amounts of [35S]methionine-labeled proteins were separated by SDS-PAGE and blotted to nitrocellulose; after transfer, filters were incubated with a monoclonal anti-HSP70 antibody (diluted 1:500; Amersham) and processed as described (10). For quantitative immunoblot analysis, after immunodetection, the individual bands were excised from the blots, and the radioactivity was determined in a scintillation counter (13).

Transcriptional Run-On Assay. In vitro run-on transcription reactions were performed in isolated K562 nuclei as described (17). 32P-labeled RNA was used for hybridization with nitrocellulose filters containing plasmids for the HSP70 gene (pH2.3; Ref. 12), kindly supplied by R. I. Morimoto (Northwestern University, Evanston, IL), or the non-HS-inducible GAPDH gene as control. After hybridization, filters were visualized by autoradiography, and the radioactivity was quantitated by a MDP analyzer (Molecular Dynamics, Sunnyvale, CA).

Northern Blot Analysis. Total cytoplasmic RNA was isolated (18), separated on 1.2% agarose/formaldehyde gels, and transferred to Hybond-N membranes (Amersham). For detection of HSP70 mRNA, the filters were hybridized with a nick-translated 32P-labeled human HSP70 gene sequence, clone pH2.3 (12). After stripping, filters were rehybridized with a 32P-labeled GAPDH probe (PstI) as a control.

Gel Mobility Shift Analysis. Whole cell extracts were prepared and binding reactions were performed using a specific 32P-labeled HSE probe as described (19). HSF-HSE complex were analyzed by nondenaturing 4% polyacrylamide gel electrophoresis. After drying, gels were processed for autoradiography, and the amount of shifted HSE probe was quantitated by MDP analysis.

RESULTS

Effect of ASA on K562 Cell Growth, Heat Resistance, and Thermotolerance. To investigate the effect of aspirin on cell proliferation and heat resistance, K562 cells were treated with different concentrations of the drug and incubated at 37°C. Concentrations of 10^{-4} M or lower did not significantly alter the rate of K562 cell proliferation, whereas higher concentrations (10^{-3} M) were found to cause an arrest of cell proliferation, as well as toxicity in long-term experiments (Fig. 1A, inset). To evaluate the effect of the drug on heat resistance, K562 cells treated with ASA or control diluent were stressed at 45°C for 15, 30, 45, or 60 min and then processed for CSA at 37°C in the absence of the drug. The exposure to a 45°C temperature resulted in a loss of viable K562 cells, which was dependent on the length of the HS (Fig. 1). Treatment with ASA had no effect on K562 heat resistance at concentrations of 10^{-4} to 10^{-5} M, whereas higher concentrations moderately inhibited the cloning efficiency in short time (15 min) treatments (Fig. 1A). The concentration of 400 μM, which did not affect either cell proliferation or heat resistance, was selected for thermotolerance studies.

To study the effect of ASA on thermotolerance, K562 cells were stressed at 45°C for 20 min and then incubated at 37°C in the presence or in the absence of ASA. At different times after the primary HS, thermotolerance was tested by exposing the cells to a second heat challenge at 45°C for 60 min, followed by CSA. Thermotolerance developed rapidly after the primary heat challenge in HS-treated cells and reached a maximum between 12 and 24 h of the recovery period at 37°C (Fig. 1B). Treatment with ASA resulted in prolonged (48–72 h) protection of K562 cells from thermal injury. In fact, whereas in control cells the surviving fraction decreased from the maximal level (approximately 100% cell survival) at 24 h to 0.5 × 10^{-2} at 48 h after the priming HS and there was no residual protection at 72 h, in ASA-treated cells the surviving fraction was still maximal at 48 h and surprisingly high even at 72 h (0.5 × 10^{-4}, Fig. 1B), to reach 1 × 10^{-3} at 96 h after the priming heat challenge. To increase thermotolerance, ASA treatment had to be started immediately after, or during plus after, the priming heat challenge. Treatment with ASA before or only during the priming HS did not modify the kinetics of thermotolerance in K562 cells subjected to lethal heating (data not shown). In the absence of a priming HS, ASA did not induce the establishment of a thermotolerant state.

ASA Modulates HSP70 Expression. The effect of ASA treatment on heat-induced thermotolerance prompted us to study the effect of this drug on HSP70 synthesis. K562 cells were stressed at 45°C for 20 min and then incubated at 37°C in the presence or in the absence of 400 μM ASA. Cells were labeled with [35S]-methionine at different times after heat shock and then processed for quantitative immunoblot

Fig. 1. Effect of ASA on K562 cell proliferation, heat resistance, and thermotolerance. A, K562 cells (2 × 10^5/ml) treated with 10^{-3} (△), 10^{-4} (●), or 10^{-5} (□) M ASA or ethanol diluent (○) were kept at 37°C, and cell numbers were determined at daily intervals (inset). Heat resistance was determined by stressing K562 cells at 45°C for 15, 30, 45, or 60 min in the presence of different concentrations of ASA. Cells were then processed for CSA in the absence of the drug. B, for thermotolerance studies, K562 cells subjected to a priming HS at 45°C for 20 min were treated with 400 μM ASA (●) or control diluent (○) during the recovery period at 37°C. At different times after the priming HS, cells were exposed to a second lethal heat challenge at 45°C for 60 min, followed by CSA. Points, mean of duplicate samples; bars, SD. Differences between control and ASA-treated cells were statistically significant at 48 and 72 h (P < 0.05). Three experiments were carried out with the same results.
A and B), causing an increase in HSP70 accumulation, as shown by quantitative immunoblot analysis (bottom). Equal amounts of protein were processed for immunoblot analysis (lop). The levels of HSP70 protein synthesized in unstressed (Q) or heat-shocked (D) cells were determined by or control diluent (-) soon after HS and labeled with [35S]methionine (5 /iCi/106 cells) for the following 24 h. Unstressed cells (- HS) were treated identically. Samples containing equal amounts of protein were processed for immunoblot analysis (top). The levels of HSP70 protein synthesized in unstressed (3) or heat-shocked (3) cells were determined by quantitative immunoblot analysis (bottom).

Fig. 2. Effect of ASA on HSP70 protein accumulation. A, K562 cells stressed at 45°C for 20 min were incubated at 37°C in the presence of 400 µm ASA (0) or ethanol diluent (C). At different times after HS, cells were labeled with [35S]methionine (1-h pulse) and processed for quantitative immunoblot analysis. Four experiments were carried out with the same results. B, cells treated with 400 µm ASA (+) or ethanol diluent (-) were either kept at 37°C (- HS) or stressed at 45°C (+ HS). Cells were labeled with [35S]methionine (1-h pulse) 5 h after HS or ASA treatment, and processed for autoradiography. Arrow, HSP70. C, K562 cells stressed at 45°C for 20 min (+ HS) were treated with 400 µm ASA (+) or control diluent (-) soon after HS and labeled with [35S]methionine (5 µCi/106 cells) for the following 24 h. Unstressed cells (- HS) were treated identically. Samples containing equal amounts of protein were processed for immunoblot analysis (top). The levels of HSP70 protein synthesized in unstressed (3) or heat-shocked (3) cells were determined by quantitative immunoblot analysis (bottom).

Analysis or autoradiography. In untreated heat-shocked cells, synthesis of HSP70 was maximal between 2 and 3 h after the shock and decreased rapidly thereafter (Fig. 2A). Treatment with ASA resulted in prolonging HSP70 synthesis for a period of several hours (Fig. 2, A and B), causing an increase in HSP70 accumulation, as shown by quantitative immunoblot analysis (Fig. 2C). In the absence of HS, ASA at this concentration did not induce HSP70 synthesis (Fig. 2B). As determined by densitometric analysis of electrophoretic patterns of [35S]methionine-labeled cells, aspirin did not appear to alter the kinetics of HSP90 and HSP110 synthesis. To investigate whether aspirin was affecting the synthesis of the low-molecular-weight HSP28, which is normally poorly labeled with L-[35S]-methionine (20), K562 cells treated as described above were labeled with L-[3H]leucine (12 µCi/106 cells, 3-h pulses) up to 9 h after heat treatment. HSP28 synthesis was detected 3 h after HS, attained maximal level between 3 and 6 h, and declined after 9 h. ASA treatment did not alter the kinetics of HSP28 synthesis (data not shown).

To determine whether the sustained synthesis of HSP70 in ASA-treated cells was due to increased HSP70 mRNA levels, in a parallel experiment, K562 cells heat shocked at 45°C for 20 min were incubated at 37°C in the presence of ASA or control diluent. At different times after HS, total cytoplasmic RNAs were isolated and processed for Northern blot analysis. In untreated heat-shocked cells, HSP70 mRNA levels were maximal between 1.5 and 3 h after the heat challenge, to go back to control level by 4.5 h (Fig. 3A). In K562 cells treated with ASA, much higher levels of HSP70 mRNA were found up to 4.5 h after HS, as compared to untreated cells (Fig. 3A). To determine whether the sustained synthesis of HSP70 protein in ASA-treated cells was due to prolonged transcription or to stabilization of HSP70 mRNA, the turnover of the HSP70 mRNA induced by a 45°C HS (20 min) was analyzed in ASA-treated cells as compared to control. The HS genes were allowed to transcribe at 37°C for 3 h after HS. The transcription inhibitor actinomycin D was then added, and the total cytoplasmic RNA was extracted at different times of further recovery and processed for Northern blot and MDP analysis. At the time of actinomycin D addition, much higher levels of HSP70 mRNA were detected in ASA-treated cells as compared to control. However, the half-life of HSP70 mRNA in ASA-treated cells was similar to control, indicating that aspirin does not increase HSP70 mRNA stability (Fig. 3B).

Effect of ASA on HSP70 mRNA accumulation and stability. A, K562 cells stressed at 45°C for 20 min were incubated at 37°C in the presence (+ ASA) or in the absence (- ASA) of 400 µm ASA. Cytoplasmic mRNA was extracted soon after HS (time 0) or at different times during the recovery period at 37°C, and processed for Northern blot analysis using 32P-labeled ph2.3 (HSP70) or PstI (GAPDH) probes. B, After HS, cells were incubated for 3 h at 37°C in the presence of 400 µm ASA (0) or ethanol diluent (C), and then treated with actinomycin D (10 µg/ml). Cytoplasmic mRNA was extracted soon after (time 0) or at 1, 2, 3, and 4 h after actinomycin D addition and processed for Northern blot analysis using 32P-labeled ph2.3 plasmid. The radioactivity was quantitated using a MDP analyzer. The values are expressed as percent of the respective control levels at time 0.

Effect of ASA on HSF DNA-binding Activity and HSP70 Transcription Rate. Sodium salicylate has been shown previously to induce HSF DNA-binding activity in HeLa cells at concentrations of 20 mM or higher (14). To determine whether aspirin could influence HSF activation, K562 cells were treated with different concentrations of ASA (4 × 10−2, 10−3, 10−4, or 10−5 M) at 37°C, and at different times after treatment, whole cell extracts were analyzed for HSF DNA-binding activity by gel mobility shift assay, using a synthetic oligonucleotide containing the consensus HSE binding site from the human HSP70 promoter. As shown in Fig. 4, also in K562 cells aspirin induced HSF-DNA binding activity in a concentration range
from 4 to 40 mM. HSF-DNA-binding activity was detected up to 4.5 h after treatment with 40 mM ASA, and up to 1.5 h with 4 mM ASA; however, lower concentrations did not induce HSF activity, even at 30 min after treatment (data not shown).

The results described in the previous section indicate that the prolonged synthesis of HSP70 protein in the presence of 400 nM ASA could be due to enhanced HSP70 mRNA transcription. To investigate this possibility and to determine whether aspirin could influence HSF activation at this concentration in heat-shocked cells, K562 cells were stressed at 45°C for 20 min and then allowed to recover at 37°C in the presence or in the absence of 400 nM ASA. Electrophoretic mobility shift assays were performed with K562 cell extracts at different times after HS. In the absence of aspirin, HSF-HSE complex formation was detected within 20 min after exposure to 45°C and continued for 1.5 h at 37°C, after which time it declined rapidly. In the presence of aspirin, the levels of HSF-HSE complex formation at 1.5 h at 37°C were higher than control, as quantitated by MDP analysis (data not shown), and HSF-DNA-binding activity was maintained up to 4.5 h after the shift to 37°C (Fig. 5A). The relative transcription rates of the HSP70 gene were examined in isolated nuclei from untreated or ASA-treated cells from the same experiment by run-on analysis. After 20 min at 45°C, HSP70 transcription rate was increased by 30-fold. After the shift to 37°C, the transcription of HSP70 gene declined rapidly in the absence of ASA, returning to basal level by 3 h, whereas in the presence of the drug, HSP70 transcription occurred at a higher rate and was prolonged for a period of approximately 3 h (Fig. 5, B and C). These results indicate that the sustained HSF-HSE complex formation in ASA-treated cells results in prolonged transcription of HSP70. According to the results described above, elevated HSP70 mRNA and HSP70 protein levels, as determined by Northern blot and quantitative immunoblot analysis, respectively, were also detected in ASA-treated cells as compared to control in the same experiment (data not shown).

The Cyclooxygenase Inhibitor INDO Mimics the Effect of ASA on K562 Thermotolerance and HS Response. ASA is a well known inhibitor of the production of arachidonic acid metabolites via the enzyme COX (15). To investigate whether the effect of aspirin on cell thermotolerance and HS response could be associated with COX inhibition, the effect of a different COX inhibitor, INDO, on heat resistance, thermotolerance, and HSP70 synthesis was studied in K562 cells.

K562 cells were treated with different concentrations of INDO and incubated at 37°C for 4 days. Concentrations lower than 10⁻⁷ M did not inhibit cell proliferation, whereas concentrations of 10⁻⁶ M or higher completely suppressed cell proliferation and were toxic in long-term experiments (data not shown). The effect of INDO on K562 cell heat resistance and thermotolerance was studied as described above for ASA. Although it did not increase heat resistance (and actually inhibited K562 cloning efficiency at the concentration of 10⁻⁶ M), treatment with 10⁻⁷ M INDO resulted in prolonging the heat-induced thermotolerant state of K562 cells with a kinetics similar to the one in ASA-treated cells (Fig. 6A).

As described above in ASA-treated cells, treatment with 10⁻⁷ M INDO in combination with hyperthermia also resulted in prolonged HSP70 transcription and translation (Fig. 6, B and C), and in prolonging the HSF-HSE-binding activity with a kinetics similar to the one described for ASA-treated cells (data not shown).

DISCUSSION

Our interest on the effect of COX inhibitors on thermotolerance and HSP synthesis derives from our recent studies on the role of prostaglandins in the HS response. We have found that cyclopentenone prostaglandins function as signals for the induction of HSP synthesis in a large variety of mammalian cells (10, 21, 22). Arachidonic acid itself was recently found to activate HSF1 and to induce the transcrip-
were incubated at 37°C in the absence (-INDO) or in the presence (+INDO) of 10^{-7} M priming HS, cells were exposed to a lethal heat challenge at 45°C for 60 min, followed by 30, 45, or 60 min, and then tested for heat resistance by CSA. For thermotolerance studies K562 cells were subjected to a priming HS at 45°C for 20 min and then incubated at 37°C in the presence (+) or in the absence (−) of 10^{-7} M INDO or ethanol diluent (O) were stressed at 45°C for 15, 20, or 30 min, and then assayed for HSP70 content by autoradiography. Experiments were performed with the same results. B, K562 cells treated with 10^{-7} M INDO. +HS, cells were exposed to a lethal heat challenge at 45°C for 60 min, followed by 30, 45, or 60 min, and then assayed for HSP70 content by autoradiography. C, K562 cells stressed at 45°C for 20 min (+HS). Cells were labeled with [35S]methionine (1-h pulse) 5 h after HS or INDO treatment and processed for autoradiography.

![Diagram](image)

Fig. 6. Effect of INDO on thermotolerance, HSP70 protein synthesis, and HSP70 mRNA accumulation in K562 cells. A (inset), K562 cells (2 x 10^5/ml) treated with 10^{-6} (A), 10^{-7} (B), and 10^{-8} (C) M INDO or ethanol diluent (C) were stressed at 45°C for 15, 30, 45, or 60 min, and then assayed for heat resistance by CSA. For thermotolerance studies K562 cells were subjected to a priming HS at 45°C for 20 min and then incubated at 37°C in the presence (+) or in the absence (−) of 10^{-7} M INDO. At different times after the priming HS, cells were exposed to a lethal heat challenge at 45°C for 60 min, followed by CSA. Points, mean of duplicate samples; bars, SD. Differences between control and INDO-treated cells were statistically significant at 48 and 72 h (P < 0.05). These experiments were performed with the same results. B, K562 cells treated with 10^{-7} M INDO (+) or ethanol diluent (−) were either kept at 37°C (−HS) or stressed at 45°C for 20 min (+HS). Cells were labeled with [35S]methionine (1-h pulse) 5 h after HS or INDO treatment and processed for autoradiography. C, K562 cells stressed at 45°C for 20 min were incubated at 37°C in the absence (−INDO) or in the presence (+INDO) of 10^{-7} M INDO. Cytoplasmic mRNA was extracted soon after HS (time 0) or at different times during the recovery period at 37°C and processed for Northern blot analysis using the HSP70 or the GAPDH probe.

The results described in the present report show that in K562 cells, ASA is able to induce HSF DNA-binding activity at concentration of 4 mM or higher. However, treatment with ASA at these concentrations completely inhibits K562 cell proliferation and is toxic in long-term experiments. At the concentration of 400 μM, which does not suppress cell proliferation, ASA does not activate HSF and does not induce HSP synthesis in the absence of hyperthermia. However, when administered during or immediately after a hyperthermic treatment, ASA causes an increase in the amount of HSP70 synthesized and prolongs HSP70 synthesis for a period of several hours longer than control. This effect is not due to increased HSP70 mRNA stability, but to an enhanced and prolonged HSP70 mRNA transcription.

It has been demonstrated that the rate of HSP70 gene transcription, HSF DNA-binding activity, and in vivo occupancy of the HSEs on the HSP70 promoter are tightly correlated during HS in human cells (27), and the decreased rate of HSP70 transcription during the attenuation phase of the HS response is also correlated with a reduction in HSF DNA-binding activity (28). We have shown that prolonged HSP70 transcription is accompanied by sustained levels of HSF-HSE complex formation, which in ASA-treated cells persists for a period twice as long as control, suggesting that ASA could be affecting an event leading to the release of bound HSF from DNA. The mechanism of down-regulation of HSF activity during the attenuation of the HS response has not been elucidated. A possible control by heat-induced phosphorylation and by interaction with negative regulatory factors has been proposed (29). The fact that a different COX inhibitor, INDO, also provokes effects similar to ASA suggests the possibility that, whereas cyclopentenone prostaglandins selectively induce HSF DNA-binding activity (12), a different product of the arachidonate cascade could be involved in the signal controlling the release of HSF from HSE. The ability of products of the arachidonate cascade to activate/inactivate protein function has been shown previously, and a mechanism of allosteric control of specific proteins by prostaglandins has been proposed (30). However, especially considering the ability of ASA at high (μM) concentrations to activate HSF also at 37°C, a different mechanism related to other activities of nonsteroidal anti-inflammatory agents, such as alterations of the cell membrane, calcium movements, and intracellular cyclic AMP levels (31), cannot be ruled out.

We have now shown that the modulation of the HS response by aspirin is associated with the ability of this drug to influence the establishment of a thermotolerant state in cells that have undergone hyperthermia. Treatment with 400 μM ASA during or immediately after a nonlethal hyperthermic treatment potentiated the effect of hyperthermia, and resulted in prolonging the interval of time in which cells are thermotolerant for at least 48 h as compared to control. INDO was also shown to prolong thermotolerance in heat-treated cells for a period twice as long as control.

Hyperthermia, alone or in combination with radiotherapy or chemotherapy, is now recognized and utilized as an effective form of treatment of certain types of cancer (2, 4, 32). The development of thermotolerance in tumor cells is one of the major concerns of...
hypothermic treatment in cancer patients. The possibility to manipulate this phenomenon to exploit a tumor-selective cytotoxicity for therapeutic gain is actively investigated. HSPs have been shown to be associated not only with resistance to hyperthermia but also with resistance to chemotherapeutic drugs, independent of the multidrug resistance system (33). On the basis of the results described, the use of ASA and INDO should be carefully monitored in cancer patients undergoing hyperthermic treatment.

The ability of ASA and INDO to prolong HSF activation and consequently HSP synthesis appears to be an unique characteristic among drugs routinely used in the clinic, especially during fever and inflammation, and could have relevant consequences. HSP synthesis is induced in mammalian brain and other organs during hyperthermia (34–36), ischemia (36,37), and exposure to neurotoxins (38), and their expression has been shown to differ in different types of tissues (39). In view of the cytoprotective role of HSPs during the pathogenesis of several disease states (40–43), the ability of ASA and INDO to enhance and prolong HSP70 synthesis could then potentiate the cytoprotective role of HSP70 in different pathological conditions, including inflammation, fever, acute virus infection, and ischemia.

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