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Herbimycin A attenuates apoptosis during heat stress in rats

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Abstract

Expression of heat shock proteins (HSPs) as a heat stress response is associated with acquisition of thermotolerance. Herbimycin A is a tyrosine kinase inhibitor that has been shown to induce HSPs. The present study aims to investigate the effects of herbimycin A on thermotolerance in rats subjected to heat stress exposure. Herbimycin A induced hsp70 to peak levels 12 h post-injection in rats without heat stress. No change in hsp70 levels was observed in the vehicle- and saline-treated rats. In rats exposed to heat stress at 45 °C for 25 min, 12 h post-treatment, lower peak temperatures were attained in herbimycin A-treated group as compared to the vehicle- and saline-treated groups. Terminal transferase-mediated d-UTP nick end labeling (TUNEL) showed that a significant decrease in apoptosis of hepatocytes in herbimycin A-treated rats as compared to the vehicle- and saline-treated rats. Caspase-3 activation was also lower in herbimycin A-treated rats, compared to the vehicle- and saline-treated rats. The present study has demonstrated that herbimycin A is effective for development of thermotolerance and therefore protects rats from heat stress.
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Keywords: Heat shock protein; Herbimycin A; Thermotolerance; Apoptosis; (Rat)

1. Introduction

Heat shock protein (HSP) induction and cell death are two distinct responses to heat stress (Pirkkala et al., 2001). Heat shock can cause cell death if cellular defense mechanisms are insufficient to cope with the stress. This is particularly obvious when the temperature increases well above that of the normal environment and/or exposure time is prolonged. An important feature of HSPs is their role in the cytoprotection and repair of cells and tissues against the harmful effects of stress and trauma. The primary mammalian hsps include proteins with molecular weights of approximately 8, 28, 58, 70, 90 and 110 kDa (Welch, 1992). HSPs consist of both stress inducible and constitutive family members. Overexpression of one or more HSP genes is sufficient to protect against otherwise lethal exposures to heat, cytotoxic drugs, toxins and tumor necrosis factor α (TNF-α) (Parsell and Lindquist, 1993). Hsp70 overexpression confers myocardial protection, as observed by resistance to myocardial ischemic stress and reperfusion damage (Mestril et al., 1994; Marber et al., 1995; Plumier et al., 1995). In inflammation, HSP was shown to protect mammalian cells from TNF-α- and β-mediated cytotoxicity (Jaattela et al., 1992), and were able to suppress astroglial-inducible nitric oxide synthase expression (Feinstein et al., 1996). In a rodent model for adult respiratory distress syndrome, heat shock-induced hsp70 accumulation within the lung has been associated with decreased pulmonary inflammation and prevention of lethality (Villar et al., 1993). The cytoprotective role of hsp70 has also been documented in the areas of metabolic disorders (Williams et al., 1993), and infection (Amici et al., 1994). These observations suggest new therapeutic strategies relying upon the development of drugs that are able to increase the expression of HSPs. Recently, it was demonstrated that HSP expression was able to protect against death following heatstroke in rats (Yang et al., 1998). It has been suggested that HSPs play a role in thermotolerance and acclimatization, and recently their use is gaining ground, both as a treatment for hyperthermia and other disease states (Morimoto and Santoro, 1998).

The objectives of this study were primarily, to find a suitable pharmacological agent that was capable of conferring thermotolerance to a rat animal model before heat stress exposure. Thermotolerance refers to an organism's ability to survive an otherwise lethal heat stress from a

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prior heat exposure sufficient to cause the cellular accumulation of HSPs. Herbimycin A was selected as the pharmacological agent of choice in this study, based on its ability to induce hsp70, without the need for a concurrent heat stress exposure (Javadpour et al., 1998; Dinh et al., 2002). An in vitro study by Dinh et al. (2002) showed that herbimycin A was able to increase survival of a human cell line following thermal injury. The use of herbimycin A in an in vivo rat model is limited to the work done by Javadpour et al. (1998), who showed that herbimycin A-attenuated ischemia reperfusion induced pulmonary neutrophil infiltration. In this study, we hypothesized that herbimycin A is able to induce hsp70 in a rat model, and subsequently protects the animal from exposure to heat stress. Parameters such as body temperature, HSP induction based on Western blots, terminal transferase-mediated d-UTP nick end labeling (TUNEL), were studied to test our hypothesis.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (250–300 g) were used in this study. The animals were handled in accordance with the guidelines of the Council for International Organization of Medical Sciences (CIOMS) ethical code for animal experimentation (Howard-Jones, 1995).

To determine the peak time of hsp70 expression, rats ($n=4$) were treated with herbimycin A and sacrificed at 6, 12, 18 and 24 h after treatment and hsp70 expression in the liver was analyzed by Western blotting. From these results, it was determined that maximum hsp70 induction occurred at 12 h post-herbimycin A treatment. Vehicle-treated rats ($n=4$) and saline-treated rats ($n=4$) were sacrificed 12 h after treatment and hsp70 expression in the liver tissues were analyzed by Western blotting for comparison. Three groups of rats were used to evaluate the effectiveness of herbimycin A in conferring thermotolerance, namely, herbimycin A-treated rats ($n=6$), vehicle-treated rats ($n=6$) and saline-treated rats ($n=6$). The animals were drug treated and exposed to heat stress 12 h later. Following heat stress, the rats were allowed to recover for 24 h, before perfusion for TUNEL staining was done.

2.2. Implantation of temperature sensor

In this study, a free moving rat model of heat stress was employed, based on an ingestible thermometric system. The ingestible sensor (CorTemp) contains a temperature-sensitive quartz crystal oscillator. The telemetered signal is inductively coupled by a radiofrequency coil system to an external receiver, attached to a clear Perspex cage, in which the rats are placed. The sensors, covered with a protective silicon coating, are 10 mm in diameter and 20 mm long and are energized by an internal silver-oxide battery. The sensors were implanted inside the peritoneal cavity.

Rats were anaesthetized with Clinical Research Center (CRC) cocktail (i.p. 0.33 ml 100/g), containing 1 part Hypnorm (Jansen Pharmaceutica, Beerse, Belgium), which contains fentanyl (0.315 mg/ml) and fluanisone (10 mg/ml), 1 part Dormicum (Roche, Basel, Switzerland), which contains midazolam (5 mg/ml), and 2 parts water for injection, before implantation. A small incision was made abdominally, above the right leg, and the sensor was placed under the skin, in contact with the peritoneal cavity. Baneocin (250 IU/bacitracin zinc. B.P., 5000 IU neomycin, as sulphate B.P., Biochemie, Vienna, Austria) was applied to the incisions, after they were closed with sutures. The operated animals were allowed to recover for 48 h, before being used in the study.

2.3. Drug treatment

One milligram of herbimycin A (Sigma) was dissolved in 1 ml of dimethylsulfoxide (DMSO), and then diluted to 6 ml with 0.9% saline. A vehicle of 1 ml of DMSO diluted to 6 ml with 0.9% saline was used. Doses of 2 ml/kg of herbimycin A solution, vehicle or saline were administered intraperitoneally (i.p.) to each of the groups. The dose of herbimycin A was selected based on that reported by Javadpour et al. (1998) where it was found to be effective in inducing hsp70.

2.4. Western blotting

At the pre-determined time points, the rats were sacrificed, and the liver was rapidly removed, washed in ice-cold saline and cut into suitable small pieces and were rapidly frozen in liquid nitrogen. The frozen tissues were stored at − 80 °C until protein extraction and quantitation was done. For the analysis of apoptosis inhibition, rats from the three treatment groups were sacrificed 24 h after heat stress.

During protein extraction, the frozen tissues were first powdered in a mortar and pestle, using liquid nitrogen. The powdered tissues were then suspended in an extraction buffer, containing phosphate-buffered saline, with 1 μg/ml of aprotinin (Sigma) and 0.01% Triton X (Bio-Rad). The tissue suspension was then homogenized and centrifuged, and the supernatant was recovered. Protein content in the supernatant was quantitated based on the Bradford assay (Bio-Rad).

Equal protein amounts of 30 μg were separated on a one-dimensional 7.5% polyacrylamide gel (Bio-Rad), under standard denaturing conditions according to the method of Laemmli (1970). Briefly, protein samples were diluted in a denaturing buffer (Laemmli, 1970), and heated at 100 °C for 5 min, and allowed to cool, before equal protein loads of 30 μg were loaded into each well. The loaded protein samples were then separated by electrophoresis. The separated proteins were then transferred onto a polyvinylidene fluoride (PVDF) membrane (Bio-Rad). The membranes were then blocked with a solution of 5% non-fat milk, probed overnight with a mouse monoclonal antibody spe-

cific for hsp70 (C92, 1:1000 dilution) at 4 °C. The C92 antibodies were obtained from StressGen Biotechnologies. After incubation overnight, the membranes were washed with Tris-buffered saline and probed with a goat anti-mouse immunoglobulin G (IgG) horseradish peroxidase-conjugated secondary antibody (1:10 000 dilution). The secondary antibodies were obtained from StressGen Biotechnologies as well. The membranes were then washed thoroughly with Tris-buffered saline, and they developed using the enhanced chemiluminescence-Western blot detection kit (Amersham-Pharmacia-Biotech) at a darkroom and exposed to X-ray film (Kodak). Densitometry analysis of the bands obtained was done using Flour S (Bio-Rad).

In the analysis of apoptosis inhibition, goat polyclonal IgG antibodies (L-18, 1:500 dilution) specific for the p20 subunit of the 32-kDa cysteine protease of caspase-3, were obtained from Santa Cruz Biotechnology and were used as the primary antibody. Bovine anti-goat IgG horseradish peroxidase from Santa Cruz Biotechnology was used as the secondary antibody. The protein samples were extracted as described above. Equal amounts of protein (30 μg) were loaded into a one-dimensional 12% polyacrylamide gel (Bio-Rad), and denatured as above. The separated proteins were then transferred onto a PVDF membrane (Bio-Rad). All the remaining steps were similarly followed as for hsp70 analysis.

2.5. Heat stress protocol

A heat stress protocol of exposure to 45 °C for 25 min at 55% humidity in a climatic chamber (Cold-Heat-Climate-Test chamber, Weiss Technik) was used in this study. Rats with the implanted sensors were allowed to move freely in a Perspex container throughout the duration of the experiments. A receiver was attached to the outside of the container to record the signals from the sensors. Following heat stress, the animals were allowed to recover at room temperature (25 °C) for another 20 min before being returned to their cages. Temperatures were recorded at 5-min intervals during the entire duration of the experiments.

2.6. TUNEL staining

Following heat stress, the rats were allowed to recover for 24 h, before perfusion was carried out. The rats were anesthetized with CRC cocktail (i.p. 0.33 ml 100/g). They were perfused with Ringer's solution followed by 10% formalin. The livers were then removed and post-fixed in a similar fixative for 24 h. They were dehydrated in an ascending series of alcohol, cleared with toluene and embedded in paraffin wax. Four-micrometer-thick coronal serial sections were cut and stained for TUNEL. The sections were stained according to the protocol for paraffin-embedded tissue provided in the TdT-FragEL™ DNA Fragmentation Detection Kit (Cat no. QIA33, Oncogene Research Products).

2.7. Statistics

SPSS version 10 was used in the analysis of the body temperatures obtained. The data from the various groups were analyzed by analysis of variance (ANOVA), followed by post hoc Bonferroni tests. Densitometry data from the Western blots were analyzed in a similar manner.

3. Results

3.1. Time expression of hsp70 in herbimycin A-treated rats

Peak hsp70 expression was seen 12 h post-herbimycin A administration, in the liver tissues. Hsp70 expression fell

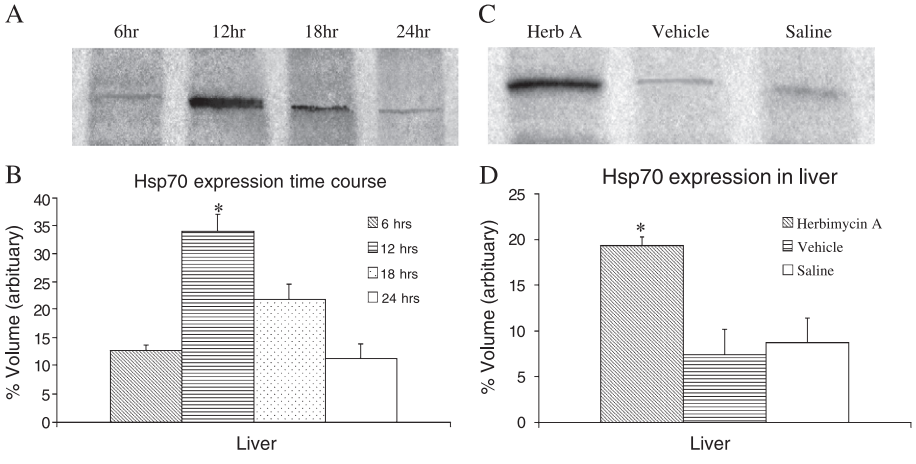


Fig. 1. (A) Western blots of hsp70, from the of herbimycin A-treated rats. The tissues were harvested at 6, 12, 18 and 24 h post-herbimycin A administration. Peak hsp70 expression, indicated by intense bands, was seen 12 h post-herbimycin A administration, in the liver tissues. (B) Densitometric analysis of hsp70 expression over time, from Western blot data, in the liver of herbimycin A-treated rats (* denotes $P < 0.05$ when compared against 6, 18 and 24 h). (C) Western blots of hsp70 from the liver of herbimycin A (Herb A)-, vehicle- and saline-treated rats. The tissues were harvested, 12 h post-drug administration. (D) Densitometric analysis of hsp70 expression from Western blot data, in the liver of herbimycin A-, vehicle- and saline-treated rats (* denotes $P < 0.05$ when compared against vehicle- and saline-treated rats).

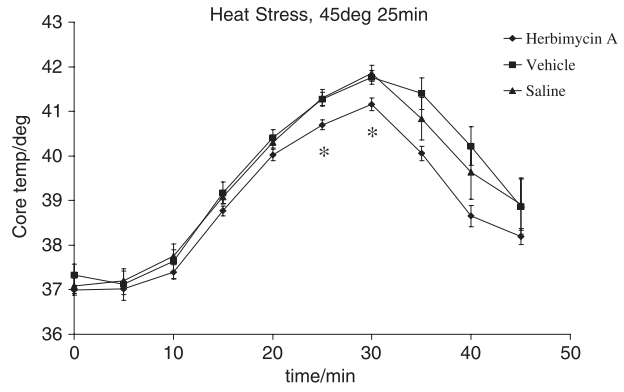


Fig. 2. Temperature time course, of rats treated with herbimycin A, vehicle and saline, exposed to 45 °C heat stress for 25 min. Each point is the mean ± S.E.M. (*: $P<0.05$ compared to YSI probe).

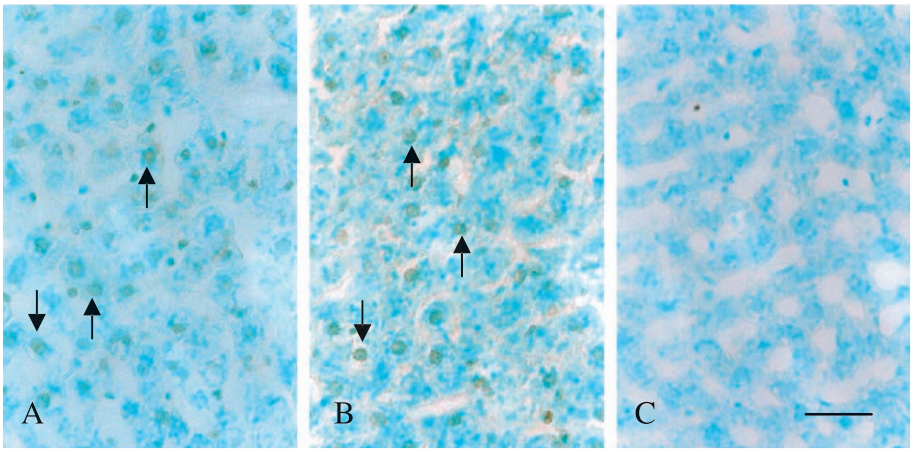
with time, as shown by the decreasing intensity of the bands at 18 and 24 h post-drug administration (Fig. 1A). Fig. 1B shows the densitometry data based on the Western blots in Fig. 1A. The data show that hsp70 expression was significantly greater at 12 h, when compared against expression at 6, 18 and 24 h ($P<0.05$).

3.2. Herbimycin A and hsp70

Herbimycin A was able to induce the expression of hsp70 in the liver tissue (Fig. 1C). Both vehicle- and saline-treated rats showed no obvious difference in hsp70 expression in the liver. Neither was able to increase the expression of hsp70 as well. Fig. 1D shows the densitometry results based on the Western blot in Fig. 1C. Expression of hsp70 was significantly greater ($P<0.05$) following herbimycin A treatment, as compared to administration of vehicle and saline, for the liver. No statistically significant differences were observed in the expression of hsp70, between vehicle- and saline-treated rats.

3.3. Heat stress and temperature

After exposure to heat stress, peak core temperatures were attained by all three groups at $t=30$ min (Fig. 2).



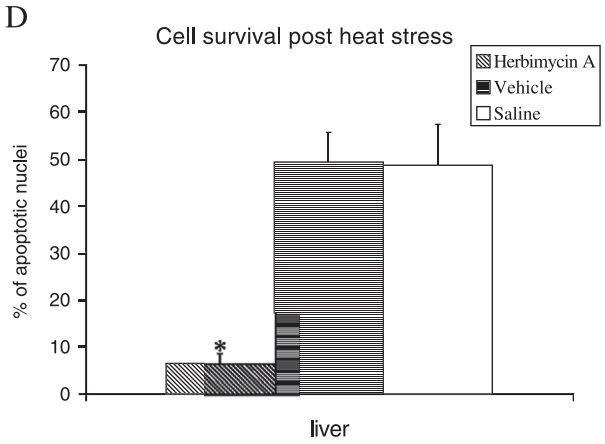


Fig. 3. (A–C) TUNEL stains of the liver from vehicle (A)-, saline (B)- and herbimycin A (C)-treated rats, respectively, 24 h after heat stress exposure. Apoptotic nuclei in the livers which are stained brown (arrows), can be clearly observed in the vehicle (A)- and saline (B)-treated rats, compared to the herbimycin A (C)-treated rat. Bar = 25 μm (for all). (D) Amount of apoptotic nuclei in the liver tissues analyzed, from herbimycin A-, vehicle- and saline-treated rats, based on the TUNEL results. The values are expressed as a percentage of the total number of cells in the respective fields (* denotes $P<0.05$ when compared against vehicle- and saline-treated rats).

Herbimycin A-treated rats attained lower peak temperatures of 41.16 ± 0.14 °C ($P < 0.05$), as compared to vehicle- and saline-treated rats that attained 41.76 ± 0.16 and 41.85 ± 0.18 °C, respectively. Herbimycin A-treated rats had lower temperatures ($P < 0.05$), as compared to vehicle- and saline-treated rats at $t = 25$ min, and as compared to vehicle-treated rats at $t = 35$ min. Both the vehicle- and saline-treated rats showed no statistically significant differences in their core temperatures throughout the duration of recording.

3.4. TUNEL staining

Apoptotic nuclei, which were stained brown, can be clearly observed in the vehicle- (Fig. 3A) and saline-treated (Fig. 3B) rat liver sections, compared to the herbimycin A-treated (Fig. 3C) rat liver sections, 24 h after heat stress exposure. A scoring system was designed whereby the total number apoptotic cells was expressed as a percentage of the total number of cells in each field, per tissue, per treatment. These data are presented in Fig. 3D. From Fig. 3D, it can be seen that the percentage of apoptotic nuclei in the tissues of the herbimycin A-treated rats was significantly lower ($P < 0.05$), as compared to that from the vehicle- and saline-treated rats. Furthermore, there was no statistically significant difference, in the percentage of apoptotic nuclei, between the liver tissues of vehicle- and saline-treated rats.

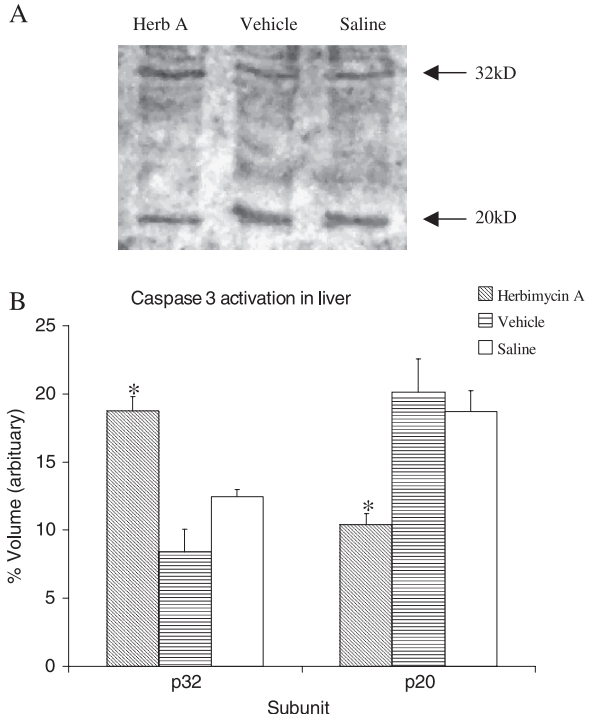


Fig. 4. (A) Western blots of caspase-3 from the liver of herbimycin A (Herb A)-, vehicle- and saline-treated rats. (B) Densitometric analysis of caspase-3 activation 24 h after heat stress, in the liver of herbimycin A-, vehicle- and saline-treated rats (* denotes $P < 0.05$ when compared against vehicle- and saline-treated rats).

3.5. Caspase-3 Western blots

Caspase-3 activation from the liver, for herbimycin A-, vehicle- and saline-treated rats was examined, 24 h after heat stress (Fig. 4A). From the Western blots, two distinct bands, at 32 and 20 kDa were observed for all three treatment groups. The 32-kDa band represents procaspase-3, and the 20-kDa band represents active caspase-3. It was observed that the procaspase-3 bands from the liver tissues of the herbimycin-treated rats were more intense, compared to the respective bands from the liver tissues of the vehicle- and saline-treated rats. Conversely, the caspase-3 bands from the liver tissues of the herbimycin A-treated rats were less intense, compared to the respective bands from the liver tissues of the vehicle- and saline-treated rats. Fig. 4B shows the densitometric data of caspase-3 activation, based on the results from Fig. 4A. Based on the densitometric data, it can be observed that procaspase-3 was present in significantly larger amounts ($P < 0.05$) in the liver tissues of herbimycin A-treated rats, as compared to the liver tissues from vehicle- and saline-treated rats. Furthermore, caspase-3 was present in lower amounts in the liver tissues of the herbimycin A-treated rats, when compared against the liver tissues of the vehicle- and saline-treated rats. No statistically significant difference was observed in the densitometric data, between the liver tissues of the vehicle- and saline-treated rats.

4. Discussion

Early studies by Murakami et al. (1991) reported that the benzoquinoid ansamycin antibiotic, herbimycin A, was capable of increasing the expression of hsp70, in a range of cells. Originally isolated as a potential herbicide (Omura et al., 1979), herbimycin A has been suggested to act as an inhibitor of tyrosine specific protein kinases (Uehara et al., 1989), via the inactivation of p-60$^{v\text{-}src}$ tyrosine kinase. Javadpour et al. (1998) demonstrated that, besides being active in cell cultures, herbimycin A was capable of inducing the expression of hsp70 in rat tissues, namely, at the gut, mesentery, pulmonary and liver tissue. They further demonstrated that herbimycin A attenuated ischemia–reperfusion-induced pulmonary neutrophil infiltration, suggesting that hsp70 induced by herbimycin A was responsible for the reduced tissue injury. In this study, the hypothesis that herbimycin A was able to induce hsp70 in rats, and subsequently protect the animals from heat stress, was tested. Western Blotting was performed on liver tissues of the rats. The liver was selected based on the findings from previous studies, which demonstrated that hsp70 rapidly accumulates in it, in response to stress exposure and hyperthermia (Flanagan et al., 1995; Skidmore et al., 1995).

The i.p. route of administration of herbimycin A to rats was investigated in this study, and based on the results obtained, it was determined that maximum hsp70 expression occurred between 12 and 18 h post-administration, in the liver. Hence, 12 h was chosen as the point of peak hsp70 expression and the subsequent experiments were timed to coincide with that point. Results from the Western blotting data of rats that were administered with saline and vehicle, showed no up-regulation of hsp70 expression following sacrifice, 12 h post-administration, when compared to herbimycin A-treated rats. Hence, the results clearly indicate that the increased expression of hsp70 observed in the rat tissues was due to the action of herbimycin A. These findings from the Western bolts support our hypothesis that herbimycin A can induce the expression of hsp70 in the liver when administered through the i.p. route.

The precise manner in which herbimycin A induces the expression of hsp70 is not known, but several postulations have been put forward to date. Hegde et al. (1995) were unable to find any evidence for the presence of any adverse effects on protein maturation or any accumulation of abnormally folded proteins in cells treated with herbimycin A. Hence, it can be concluded that the induction of hsp70 by herbimycin A is through another mechanism, other then protein damage. Herbimycin A did not induce hsp90, 60 or 25 in rat neonatal cardiac cells, unlike in thermal preconditioning, where the various HSPs were induced (Morris et al., 1996). This led Morris et al. (1996) to propose that the mechanism of hsp70 induction by herbimycin A may not occur via the activation of the heat shock factor, unlike the agents or mechanisms that induce the heat shock response (Minowada and Welch, 1995). Instead, herbimycin A may act via a distinct and possibly less ‘‘stressful’’ pathway for hsp70 induction. This proposal was further supported by the fact that herbimycin A appeared to induce both hsp70 and hsc70 strongly, and this contrasts with the pattern of Hsp70 induction evoked by the stress response. To determine if the hsp70 induction by herbimycin A was related to its tyrosine kinase inhibitory properties, Morris et al. (1996) employed another tyrosine kinase inhibitor, genistein, under similar conditions. Despite utilizing a range of doses that were adequate for tyrosine kinase inhibition, genistein was unable to induce any HSPs and was similarly unable to protect the cardiomyocytes from lethal stress, unlike herbimycin A. Hence, the tyrosine kinase inhibitory activity of herbimycin A is unlikely to be responsible for its action with regard to hsp70 induction and enhanced tolerance against lethal stress.

It is well known that heat stress causes apoptosis, or programmed cell death by a process involving early nuclear factor kappa b (NF-κB) nuclear translocation and caspase-3 activation. It has been shown that apoptotic nuclei in tissues can be detected as soon as 12 h post-stress exposure, and decline to basal levels within 48 h (Sakaguchi et al., 1995). In agreement with this, the present results have also shown widespread apoptotic nuclei present in the liver sections of the vehicle- and saline-treated rats. However, only sparingly distributed apoptotic nuclei were observed in the liver tissues of the herbimycin A-treated rats. It is clearly evident that herbimycin A was able to reduce the percentage of apoptotic nuclei significantly following heat stress, as compared to vehicle- and saline-treated rats. The Western blot results based on caspase-3 activation show that procaspase-3 was cleaved to active caspase-3 to a greater extent in the tissues of the vehicle- and saline-treated rats, compared to the respective liver tissues of the herbimycin A-treated rats. This could attribute to the higher level of apoptotic nuclei observed post-heat stress in the tissues of the vehicle- and saline-treated rats. The presence of the overexpression of hsp70 in the liver tissues of herbimycin A-treated rats could have been responsible for the inhibition of apoptosis in these tissues. It was proposed that hsp70 could offer protection to cells from energy deprivation and/or ATP depletion associated with cell death (Wong et al., 1998). Through the generation of transient or stable transfections, it was shown that elevated levels of hsp70 reduced or blocked caspase activation and hence suppressed mitochondrial damage and nuclear fragmentation (Buzzard et al., 1998). These findings were reinforced by the discovery that hsp70 inhibited apoptosis downstream of the release of cytochrome *c* and upstream of caspase-3 activation (Li et al., 2000). It was proposed that this inhibition was via a hsp70-mediated modulation of the apoptosome. Furthermore, hsp70 was shown to directly bind to Apaf-1, hence preventing the recruitment of procaspase-9 to the apoptosome (Saleh et al., 2000; Beere et al., 2000). Hsp70 was shown to prevent morphological changes downstream of caspase-3 activation that are characteristic of dying cells (Jaattla et al., 1998). It was also proposed that hsp70 interacted with the apoptotic pathway at early steps, such as by preventing JNK activation (Meriin et al., 1999). Indeed it was shown that hsp70 binds to and functions as a natural inhibitory protein of c-Jun N-terminal kinase (JNK1) (Park et al., 2001). Through these mechanisms, hsp70 may play an important role for cell protection.

Besides HSP induction, it has been suggested that herbimycin A can exert its cytoprotection through other mechanisms since HSP nuclear translocation does not always occur with herbimycin A induction (Morris et al., 1996). Gene expression profiling by Dinh et al. (2002) indicated that in addition to the induction of the classic heat shock genes (i.e., hsp90, hsp70), transcription for a variety of other genes encoding growth factors and transcription factors were up-regulated and may be involved in protection by herbimycin A. Nishiya et al. (1995) have suggested that herbimycin A may directly modify the transcription factor, NF-κB (Nishiya et al., 1995). Thus, herbimycin A appears to protect against thermal-induced cell death by inducing HSPs and blocking nuclear translocation of NF-κB in a process that attenuates caspase-3 activation.

Temperature data obtained from the free moving heat stress model, during the evaluation of herbimycin A, showed that herbimycin A-treated rats achieved statistically significant lower peak core temperatures, compared to the

saline- and vehicle-treated rats. The mechanism by which herbimycin A was responsible for the lower peak core temperatures was not clear. A likely mechanism may be related to the release of endotoxins from the gastrointestinal tract and cytokine production, following exposure to heat stress, as proposed by Moseley (1997). Several studies have demonstrated the presence of systemic endotoxemia (Bosenberg et al., 1988; Bouchama et al., 1991) and elevations in circulating levels of cytokines with heat exhaustion and heat stroke. Strenuous exercise has also been demonstrated to elicit a cytokine response (Cannon and Kluger, 1983; Villar et al., 1994). It was postulated that the increases in cytokine levels could take place in response to circulating endotoxins, which could have translocated across the gut barrier, due to heat-related alterations in gut permeability (Hall et al., 2001). Interestingly, elevations in cellular hsp70 were found to be associated with attenuation in heat-induced permeability of the epithelial monolayer (Moseley et al., 1994). The preservation of the epithelial barrier through an HSP-associated mechanism, possibly through the stabilization of the cytoskeleton or through the preservation of important cell to cell contacts, may be an important factor in preventing heat-associated endotoxin translocation across the gut. Furthermore, animals that were made to undergo a conditioning stress sufficient to cause hsp70 accumulation showed a decrease in circulating TNF-α after endotoxin exposure (Kluger et al., 1997). Hence, the ability of herbimycin A to attenuate the peak temperatures attained after heat stress exposure could be related to the effects of increased expression of hsp70, as described above.

In conclusion, the present study has demonstrated that herbimycin A is capable of inducing hsp70 which could have played a very likely role in the thermoprotection. Rats with high levels of hsp70 in their tissues were able to attain lower peak core temperatures following heat stress, and also demonstrated a lower percentage of apoptotic nuclei in the liver. This study has confirmed that herbimycin A can be used effectively to protect rats exposed to heat stress. Whether it can carry out a similar effect in humans is yet to be determined, but if so, it would provide a tremendous opportunity to exploit the potentially beneficial effects of hsp70 up-regulation in the prevention and treatment of disease conditions.

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