Heat shock protein 90-binding agents protect renal cells from oxidative stress and reduce kidney ischemia-reperfusion injury

Ewen M. Harrison,1 Eva Sharpe,1 Christopher O. Bellamy,2 Stephen J. McNally,1 Luke Devey,1 O. James Garden,1 James A. Ross,1 and Stephen J. Wigmore1

1Tissue Injury and Repair Group and 2Department of Pathology, University of Edinburgh, Edinburgh, United Kingdom

Submitted 2 August 2007; accepted in final form 12 June 2008

Heat shock protein 90-binding agents protect renal cells from oxidative stress and reduce kidney ischemia-reperfusion injury. Am J Physiol Renal Physiol 295: F397–F405, 2008. First published June 18, 2008; doi:10.1152/ajprenal.00361.2007.—Heat shock proteins (Hsps) are protective in models of transplantation, yet practical strategies to upregulate them remain elusive. The heat shock protein 90-binding agent (HBA) geldanamycin and its analogs (17-AAG and 17-DMAG) are known to upregulate Hsps and confer cellular protection but have not been investigated in a model relevant to transplantation. We examined the ability of HBAs to upregulate Hsp expression and confer protection in renal adenocarcinoma (ACHN) cells in vitro and in a mouse model of kidney ischemia-reperfusion (I/R) injury. Hsp70 gene expression was increased 30–40 times in ACHN cells treated with HBAs, and trimerization and DNA binding of heat shock transcription factor-1 (HSF1) were demonstrated. A three- and twofold increase in Hsp70 and Hsp27 protein expression, respectively, was found in ACHN cells treated with HBAs. HBAs protected ACHN cells from an H2O2-mediated oxidative stress, and HSF1 short interfering RNA was found to abrogate HBA-mediated Hsp induction and protection. In vivo, Hsp70 was upregulated in the kidneys, liver, lungs, and heart of HBA-treated mice. This was associated with a functional and morphological renal protection from I/R injury. Therefore, HBAs mediate upregulation of protective Hsps in mouse kidneys which are associated with reduced I/R injury and may be useful in reducing transplant-associated kidney injury. Heat shock, or stress, proteins are a highly conserved group of intracellular chaperones that are expressed when a cell is exposed to one of an array of metabolic insults, including heat, oxidative stress, hypoxia, ischemia, and heavy metals (28). Increased heat shock protein expression following stress is associated with a tolerance to subsequent injury that would otherwise be lethal (12). Heat shock protein upregulation has been associated with cytoprotection in many models of cellular stress and has great potential for future clinical intervention (for a recent review, see Ref. 4). One area of promise is solid organ transplantation. Heat-induced expression of stress proteins has been associated with protection in rat models of kidney (42, 43), liver (26, 29) and lung (18) transplantation. Increased heat shock protein 70 (Hsp70) expression by gene transfer has improved the outcome in rat lung isografts (17) and reduced I/R injury following rat heart transplantation (20). Much interest has been shown lately in the small heat shock protein heme oxygenase-1 (HO-1; Hsp32) (for a review, see Ref. 22), which has been associated with an improvement in the outcome in heat-treated rats subjected to liver (29, 41) and kidney transplantation (51).

The stress protein response is regulated by heat shock transcription factor-1 (HSF1) and transcriptional activation requires HSF1 trimerization, nuclear localization, and hyper-phosphorylation. An important component regulating transcription activation appears to be the loss of a repressive multichaperone complex (50). The proteins Hsp90 and p23 form a complex with HSF1 preventing it from becoming transcriptionally active. Increased concentrations of unfolded proteins following stress competitively bind Hsp90 (and other chaperones), releasing HSF1 and allowing it to become transcriptionally active. Thus interference with the formation of this complex represents a potential mechanism by which the stress response can be activated.

The identification of geldanamycin (GA) marked the first description of a group of drugs termed the benzozquinone ansamycins. Antitumor activity was demonstrated (49), which was later shown to be due to Hsp90 inhibition (53). The benzozquinone ansamycins bind to the ATP-binding site of Hsp90, thus preventing intrinsic ATPase activity (46). A number of studies have investigated the use of Hsp90-binding agents (HBAs) in the modification of HSF1 activity. GA was first shown to reduce the amount of HSF1-bound Hsp90 in vitro (33) and to delay recovery from heat shock and increase HSF1 DNA-binding in vivo (1, 58). Hsp90 inhibition and consequent Hsp upregulation have been shown to be protective following heat stress (6, 8, 16, 30, 44), simulated ischemia (6, 14, 30, 55), I/R injury (19, 24), and hemorrhagic shock (40), but none of these studies are directly relevant to transplantation. GA, the most promising early HBA, has been shown to have unacceptable side effects in preclinical trials, and less toxic analogs have been sought (47). Two such analogs, 17-allylaminoo-17-demethoxygeldanamycin (17-AAG) and 17-((dimethylaminoethyamino)-17-demethoxygeldanamycin (17-DMAG) have been shown to have good Hsp90 inhibition in vitro, but they have not been examined in models of cellular

Address for reprint requests and other correspondence: E. M. Harrison, Tissue Injury and Repair Group, Univ. of Edinburgh, Rm. FU501, Chancellor’s Bldg., Little France Crescent, Edinburgh EH16 4SB, UK (e-mail: mail@ewnarrison.com).
protection. HBAs have antineoplastic effects, and both 17-AAG and 17-DMAG have been shown to have significantly lower systemic side effects than geldanamycin in phase 1 (21) and preclinical trials (13), respectively.

In view of the beneficial effects of upregulation of stress proteins in models of organ transplantation, we wished to determine whether these new HBAs could protect renal cells against oxidative stress and act to reduce I/R injury in mouse kidneys.

MATERIALS AND METHODS

Cell culture and reagents. Human renal adenocarcinoma cells (ACHN; European Collection of Cell Cultures, Porton Down, UK) were maintained as previously described (15). Reagents were purchased from Sigma-Aldrich (Poole, Dorset, UK) unless otherwise stated. The Hsp90 HBAs GA, 17-AAG, and 17-DMAG were purchased from InvivoGen (San Diego, CA). HBA stock solutions (10 mg/ml in DMSO were further diluted to 100 μg/ml with 0.9% saline before ip administration). Antibodies were the following: rat anti-Hsp90 monoclonal (16F1 clone), rabbit anti-Hsp70 polyclonal (Hsp72; inducible; used for in vivo Western blots) mouse anti-Hsp70 monoclonal (Hsp72; inducible; C92 clone; used for in vitro Western blots), rabbit anti-Hsp27 polyclonal, rabbit anti-Hsp25 polyclonal, and rabbit anti-HSF1 were obtained from Stressgen (Victoria, BC, Canada); mouse anti-β-actin monoclonal antibody was from BD Biosciences (San Diego, CA).

Animals. Male BALB/c mice, aged 6 – 8 wk and weighing 20 – 30 g, were purchased (Harlan Laboratories, Blackthorn, UK) and caged for a minimum of 5 days before experimentation. Animals were allowed free access to standard chow and water and kept in a 12:12-h light-dark cycle. All work involving animals was conducted in accordance with the provisions of the UK Animals (Scientific Procedures) Act 1986, and institutional ethical approval was obtained.

Fig. 1. Heat shock protein induction and protection from H2O2 by heat shock protein 90-binding agents (HBAs) in renal adenocarcinoma (ACHN) cells. ACHN cells were treated with 2 μM geldanamycin (GA), 17-allylamino-demethoxygeldanamycin (17-AAG), or 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) for various times (A and B) or increasing concentrations of GA, 17-AAG, or 17-DMAG for 8 h (C). Whole-cell lysates were prepared and analyzed by Western blotting using antibodies to Hsp90, Hsp70, and Hsp27, with β-actin being indicated as a loading control. D: ACHN cells were treated for 1 h with 2 μM GA, 17-AAG, 17-DMAG, vehicle (V), or heated to 43°C for 45 min (HS). Nuclear extracts were obtained, and a DNA mobility shift assay was performed using γ-32P-labeled heat shock transcription factor-1 (HSF1) oligonucleotides. Control reactions were performed that included a 20 μl molar excess of unlabeled consensus sequence (CP) or an antibody to HSF1 (Shift). E: ACHN cells were treated with GA, 17-AAG, 17-DMAG, or vehicle (V) for 2 h, after which mRNA extracts were prepared using TRIzol and reverse transcribed to cDNA. Fluorescence-detected real-time PCR was performed using Hsp70 primers and probe with an 18S primer/probe control; results are expressed as mean expression relative to vehicle ± SE of 3 independent experiments. F: ACHN cells were pretreated for 8 h with GA, 17-AAG, 17-DMAG, or vehicle control (DMSO), after which the medium was removed and replaced by medium containing 2 μM hydrogen peroxide (H2O2) for 16 h. An MTT assay was performed, with results expressed as the relative mean cell viability ± SE of 3 independent experiments. * P < 0.05, ** P < 0.01 compared with vehicle (DMSO; 1-way ANOVA with Tukey’s post hoc correction).
Renal I/R model. A model of warm I/R injury of the left kidney and unilateral nephrectomy was adapted (38). The model was designed to inflict a moderate acute tubular injury while minimizing animal mortality. Mice were anesthetized with an intraperitoneal injection of ketamine (75 mg/kg, Vetalar; Pfizer, Kent, UK) and medetomidine (1 mg/kg, Domitor, Pfizer). A subcutaneous injection of 0.9% saline (25 ml/kg) and buprenorphine (0.1 mg/kg) was administered before the procedure, and a thermostatically controlled heated mat was used to maintain body temperature. The pedicle of the left kidney was dissected and occluded using an atraumatic vascular clamp (6 mm, Micro Серрефіні, Fine Science Tools, Linton, UK) for 30 min. Meanwhile, the right kidney was dissected and a titanium clip was placed across the ureter and the renal pedicle (Hemoclip Plus, Weck Closure Systems, Research Triangle Park, NC), after which the kidney was removed as a control. Following removal of the left pedicle clamp, reperfusion was confirmed visually before closure of the abdomen. The anesthetic was reversed with atipamezole (1 mg/kg, Antisedan, Pfizer), and a further 25 ml/kg of 0.9% saline was given 2 h after the procedure. Animals were recovered in an incubator at 25°C or a metabolic cage (Tecniplast, Exton, PA) depending on the protocol.

Tissue collection. Under terminal general anesthesia, blood was recovered by intracardiac puncture, and the serum was stored at −20°C. The left kidney was divided equally in the transverse plane, with one portion snap-frozen in liquid nitrogen and the other being placed in methacarn (70% methanol, 20% chloroform, 10% acetic acid). The liver, lungs, and heart were removed and placed in liquid nitrogen. All frozen tissue was maintained at −70°C until analysis.

Western blotting. Western blotting was performed as previously described (15). Briefly, whole-cell extracts were produced and protein concentration determined. Proteins were separated by SDS-PAGE (10% Tris·HCl gels), transferred to nitrocellulose membranes (Bio-Rad), and exposed to a blocking buffer and the primary antibody. Horseradish peroxidase-conjugated secondary antibody was used at a concentration of 1:5,000. Enhanced chemiluminescence reagent (Amersham, Chalfont St. Giles, UK) was applied and development by autoradiography performed.

DNA mobility-shift assay. Nuclear lysates were prepared using Gobert’s method (1). DNA mobility-shift assay was performed as previously described (37). Briefly, forward (5’-ATCTCCGGCTGAAATATTCCCGACTCGGCAAGCAGGA) and reverse (5’-GATCTCGGCTGACCGATGGAATATTCCAGCGG) oligonucleotides specific for HSF1 were radio-labeled with [γ-32P]ATP. Ten micrograms of protein was incubated with 0.25 ng labeled probe in the presence of poly [d(I-C)] (Sigma). Control reactions were performed that included a 20× molar excess of unlabeled consensus sequence or 1 μl anti-HSF1 antibody. Reactions were run on 5% TBE polyacrylamide gels (Bio-Rad) and exposed by autoradiography.

RNA isolation and fluorescence detection real-time PCR. RNA extraction and purification was performed using TRIzol (Invitrogen, Paisley, UK). RNA was DNase treated and reverse transcribed to cDNA using avian myeloblastosis virus reverse transcriptase (Promega) and random decamers (Ambion, Huntington, UK). Fluorescence-detected real-time PCR was then performed using Hsp70-specific primers and a TAMRA-labeled FAM probe. Samples were run on an ABI Prism 7700 Sequence Detection System and analyzed using Sequence Detector 7.1 (Applied Biosystems).

Cell viability assay. Cells were treated as per the experimental protocol in 96-well plates. Twenty microliters of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (5 mg/ml) was added for 2 h. Wells were then carefully emptied, and the tetrazolium crystals were dissolved in 10% SDS (pH 3.0). Samples were analyzed on a plate reader (test: 570 nm; reference: 630 nm) with increasing optical density reflecting an increasing number of viable cells.

RNA interference. Cells were seeded in six-well tissue culture plates using antibiotic-free media supplemented with 10% fetal calf serum, and 50% confluence was achieved 24 h later. HSF1 siRNA (h; Santa Cruz) or control siRNA (30 pmol) was diluted in 100 μl of serum/antibiotic-free medium and 3 μl siRNA Transfection Reagent (Santa Cruz) diluted in a further 100 μl of serum/antibiotic-free medium. These were combined and incubated at room temperature for 30 min. Cells were transfected to a total volume of 1 ml serum/antibiotic-free medium. Twenty-four hours later, 1 ml medium with 20% fetal calf serum was added to each well. Twenty-four hours later, cells were treated as per the experimental protocol. Adequacy of effect was ascertained with Western blot analysis with anti-HSF1 antibody.
Hsp70 ELISA. Hsp70 expression in different mouse tissue following treatment with HBAs was quantified using a commercially available Hsp70 ELISA kit (Stressgen) as per the manufacturer’s instructions.

Immunohistochemistry. Immunohistochemistry performed was as previously described (37). Briefly, endogenous peroxidase activity was blocked with 3% hydrogen peroxide (H2O2) and antigen retrieval was performed in boiling 10 mM citrate buffer (pH 6.0). Sections were blocked in 10% normal rabbit serum, exposed to primary antibody (1:250 dilution), followed by a peroxidase-conjugated secondary antibody and a peroxidase substrate solution (3,3-diaminobenzidine tetrahydrochloride, DAKO, Glostrup, Denmark). Appropriate primary antibody-only and secondary antibody-only controls were always performed to exclude nonspecific staining.

Scoring of morphological injury. Hematoxylin- and eosin-stained sections were evaluated for tubulointerstitial injury in the outer stripe of the outer medulla (OSOM). Sixteen nonoverlapping fields (×200) were examined by two independent observers (C. O. Bellamy and E. M. Harrison). A semiquantitative scoring system was adapted from previous descriptions (34). Two scores were determined: the first was based on the proportion of tubules exhibiting dilatation and/or a loss of the epithelial brush border (tubular damage score), and the second was based on the proportion of tubules with necrotic/detached cells (tubular necrosis score: 0, none; 1, <30%; 2, 30–70%; 3, >70%).

Serum urea and creatinine determination. Serum was analyzed using an automated Olympus AU 2700 Clinical Chemistry System.

Statistics. A power calculation was performed to determine required group sizes for the renal I/R injury experiments. In a previous study, Hsp70 induction by a different method protected mice from 30-min bilateral I/R injury (48). An effect size of 1.6 was estimated based on serum urea and creatinine levels at 24 h, which equated to 10 animals/group (α = 0.05, power = 0.9) (11). Data are presented as means ± SE. Statistical comparisons were made using one-way and two-way (without interaction) ANOVA with the Tukey’s post hoc correction for multiple comparisons. Histological injury scores were compared using the Mann-Whitney U-test. All statistical analyses were performed using SPSS v14.0 (SPSS, Chicago IL).
RESULTS

HBAs induce heat shock proteins in ACHN cells. The effects of different HBAs in vitro were examined in a human renal adenocarcinoma cell line (ACHN). A time course using GA, 17-AAG, and 17-DMAG (all 2 μM) demonstrated a 3.5-fold increase in Hsp70 expression after 6 h (Fig. 1A). Hsp90 expression was 3.4-fold greater 4 h after treatment with GA and 2.5-fold greater 4 h after 17-AAG or 17-DMAG. The small heat shock protein Hsp27 was not found to be upregulated at these early time points; however, in 17-DMAG (2 μM)-treated cells there was a twofold increase at 12 h, with the same increase at 16 h in cells treated with GA or 17-AAG (both 2 μM; Fig. 1B). The maximal dose-response of Hsp70 and Hsp90 was seen with HBAs at a concentration of 0.2 μM (Fig. 1C). HO-1 was not found to be induced by HBAs in ACHNs (data not shown). Cells treated for 1 h with HBAs demonstrated trimerization of HSF1 on a DNA mobility-shift assay (Fig. 1D). Hsp70 gene expression was increased 30-, 35-, and 42-fold following 2 h treatment with GA, 17-AAG, and 17-DMAG, respectively (all 2 μM; Fig. 1E).

HBAs protect ACHN cells from oxidative stress. Pretreatment of cells with HBAs protected them from a H2O2-mediated oxidative insult (Fig. 1F). This protection did not reach statistical significance after 8 h of GA (2 μM) but was significant in groups treated with 17-AAG (P < 0.05) and 17-DMAG (P < 0.01).

HSF1 siRNA abrogates HBA-mediated heat shock protein induction and protection. To determine whether HBA-mediated heat shock protein expression was HSF1 dependent, cells were transfected with HSF1 short interfering RNA (siRNA). Forty-eight hours later, cells were treated with HBAs for 8 h, and heat shock protein induction was determined (Fig. 2A).
HSF1 Western blot confirmed that adequate HSF1 silencing was consistently achieved. When control siRNA-transfected cells were treated with HBAs, a two- to threefold increase in Hsp70 protein was found. This increase was reduced when cells were transfected with HSF1 siRNA. The viability of cells transfected with siRNA and exposed to H$_2$O$_2$ was lower than that of cells exposed to H$_2$O$_2$ alone, although this difference did not reach statistical significance (Fig. 2B). Similar levels of protection were seen in H$_2$O$_2$-treated cells transfected with control siRNA and pretreated with HBAs, compared with untransfected HBA-treated cells. This protection was lost in HSF siRNA cells pretreated with HBAs and exposed to H$_2$O$_2$; this did not achieve statistical significance in the GA group, but was significant in the 17-AAG and 17-DMAG groups ($P < 0.05$).

HBAs induce heat shock proteins in mice. BALB/c mice were given an intraperitoneal injection of HBAs and killed at various times. No toxic effects were seen in treated mice over a 2-wk period following HBA treatment. Neither Hsp90 nor HO-1 was induced by the HBAs in mice (Fig. 3A). A two- to threefold increase in Hsp70 was seen following HBA administration. This was maximal at 6 h in mice treated with 17-DMAG, which was significantly earlier than in GA- or 17-AAG-treated groups. A twofold increase in Hsp25 was seen which, again, occurred significantly earlier in the 17-DMAG group (6 h) compared with the GA and 17-AAG group. Hsp70 and Hsp25 induction was maintained in all groups until at least 24 h. An ELISA for Hsp70 was performed on whole-organ lysates. Significant induction of Hsp70 was seen in the liver and lung following HBA administration (Fig. 3B). A threefold increase was seen in the liver which became statistically significant at 8, 16, and 6 h for GA, 17-AAG, and 17-DMAG, respectively. A two- to threefold increase in Hsp70 was found in the lungs which was significant at 6 h in 17-AAG and 17-DMAG groups. In lungs, there was a drop-off of Hsp70 levels between 8 and 16 h. In the heart, Hsp70 was found to be expressed in animals that had been treated with HBAs for 6 or 8 h. Hsp70 levels at all other time points were undetectable.

Fig. 5. Renal injury following treatment with HBAs and renal pedicle clamping ($n = 50$). BALB/c mice were pretreated with an intraperitoneal injection of vehicle (A; $n = 10$), GA (B; $n = 10$), 17-AAG (C; $n = 10$) or 17-DMAG (D; $n = 10$; all 1 mg/kg). After 16 h the left renal pedicle was clamped for 30 min, during which the right kidney was removed. Twenty-four hours later, the animals were killed and the left kidney was placed immediately in methacarn. Sections were stained with haematoxylin and eosin and show the outer stripe of the outer medulla (arrow, A, B, C, and D). The tubular damage (E) and necrosis scores (F) were determined by 2 independent observers. *$P < 0.05$, **$P < 0.01$ compared with vehicle (Mann-Whitney U-test).
Immunohistochemistry was performed to localize the expression of Hsp70 and Hsp25. BALB/c mice were injected with GA (results not shown), 17-AAG (results not shown), 17-DMAG (Fig. 4, E–H and M–P) (all 1 mg/kg) or vehicle (Fig. 4, A–D and I–L). In vehicle-treated mice at 16 h, Hsp70 immunopositivity was maximal in the tubular epithelial cells of the inner medulla/papilla (large arrow, Fig. 4C). Significant immunopositivity was also seen in the outer medulla (Fig. 4, A and D), with much lower levels in the cortex (Fig. 4, A and B). Following treatment with HBAs, Hsp70 immunopositivity was increased in the cortex (Fig. 4, E and F) but most strongly increased in the OSOM (small arrow, Fig. 4, G and H). Hsp25 was found to be expressed at lower levels than Hsp70, which is consistent with the Western blot findings (Figs. 3A and 4, I–L). Following treatment with HBAs, immunopositivity increased in the tubular epithelium of the OSOM (small arrow, Fig. 4, M and P) and to a lesser extent in the cortex (Fig. 4N).

**HBAs reduce renal damage following I/R injury.** BALB/c mice were treated with GA, 17-AAG, 17-DMAG, or vehicle and subjected to 30 min of left renal pedicle clamping 16 h later. After 24 h, animals were killed and hematoxylin- and eosin-stained kidney sections were examined and scored by two independent observers (Fig. 5, A–D). Histological injury following left renal pedicle clamping was less severe in animals pretreated with HBAs, particularly in the OSOM (small arrow, Fig. 5, A–D). This was scored in a semiquantitative manner. The tubular damage score was reduced in mice treated with HBAs and was statistically significant in the 17-AAG and 17-DMAG groups (Fig. 5E; P < 0.05). The tubular necrosis score was reduced in mice treated with HBAs and was significant in all groups (Fig. 5F; P < 0.01). Serum urea and creatinine were significantly increased following 30 min I/R injury and contralateral nephrectomy (Fig. 6, A and B). In mice pretreated with HBAs, both urea and creatinine were found to be lower 24 h after I/R injury than vehicle-treated groups. This was significant in the 17-AAG (P < 0.05) and 17-DMAG (P < 0.01) groups. There was a significant difference in serum urea between those treated with GA and the 17-DMAG-treated group (P < 0.05).

**DISCUSSION**

Heat shock proteins are known to reduce the effects of I/R injury in models of transplantation, yet practical strategies of upregulation that may be implemented clinically remain elusive. In this study, we have demonstrated that treatment with the HBAs GA, 17-AAG, and 17-DMAG results in increased expression of Hsp70 and Hsp25/27 in human cells in vitro and murine cells in vivo. This is associated with cell protection from oxidative injury in vitro and less morphological and functional injury following I/R injury in a mouse model which included unilateral nephrectomy.

Little has been published about Hsp induction in the kidney following HBA treatment. Treatment of rats with two intraperitoneal doses of GA (1 mg/kg) has been shown to marginally induce renal Hsp70 (40). GA has been consistently shown to induce Hsps in other tissues, but less is known about Hsp induction following 17-AAG or 17-DMAG. 17-AAG induces Hsp70 in colorectal cancer cells (5), primary glial cultures (7), and motor neurons (2, 52), and a phase 1 clinical trial in patients with advanced solid tumors found Hsp70 induction in peripheral blood mononuclear cells (35). In SCID mice bearing breast cancer tumors, 17-DMAG (75 mg/kg iv) increased renal and liver Hsp70 after 7 h, while Hsp90 was not induced (10).

Hsp70 and Hsp25/27 induction was significantly earlier following 17-DMAG than the other HBAs (Figs. 1, A and B and 3, A–C). 17-DMAG-treated cells also appeared to exhibit higher levels of Hsp70 mRNA at 2 h (Fig. 1F) and provide better protection against H2O2 (Fig. 1E). Although our protocol was not powered to demonstrate potential differences between the HBAs, the serum urea of 17-DMAG-pretreated animals was significant in the OSOM (small arrow, Fig. 4, M and P) and to a lesser extent in the cortex (Fig. 4N).

The baseline distribution of Hsp25 and Hsp70 was found to be similar to previous descriptions (32, 45) (Fig. 4). HBA treatment resulted in significant Hsp70 induction in the cortex but was most striking in the OSOM (Fig. 4H). This is partic-
ularly significant as this site is most susceptible to ischemic injury. The increase in Hsp25 was less pronounced but still apparent, particularly in the OSOM (Fig. 4, M and P).

In an effort to establish whether the HBA-mediated Hsp expression was HSF1 dependent, cells were transfected with HSF1 siRNA. These experiments were hampered by the significant stress response and cell death observed following transfection using many of the commercial siRNA systems tested. Hsp70 induction and cell death were minimized by reducing siRNA/transfection reagent volumes and accepting a lower level of knockdown. The results of these experiments suggest that HBA-mediated Hsp70 induction occurs in an HSF1-dependent manner. This is supported by the observations that although HSF1−/− mice express constitutive Hsps, the Hsp response to heat is completely abolished (27, 54, 57) and is greatly attenuated following endotoxin (23). Differential the Hsp response to heat is completely abolished (27, 54, 57).

REFERENCES

GRANTS

This study was funded by grants from the British Transplantation Society, Tenovus Scotland, the Scottish Hospital Endowment Research Trust (SHERTR) Medical Research Scotland, and the Mason Medical Research Foundation. E. M. Harrison has been supported by a fellowship from the British Transplantation Society supported by Novartis Pharmaceuticals.

ACKNOWLEDGMENTS

The authors thank Kathryn Sangster, Jim Black, and Ian Ansell for technical support.

REFERENCES


