The role of heat shock protein 90 in modulating ischemia–reperfusion injury in the kidney

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Introduction: Kidney transplantation is the gold standard treatment for end-stage renal disease. Ischemia–reperfusion injury (IRI) is an unavoidable consequence of the transplantation procedure and is responsible for delayed graft function and poorer long-term outcomes.

Areas covered: Pharmacological induction of heat shock protein (Hsp) expression is an emerging pre-conditioning strategy aimed at reducing IRI following renal transplantation. Hsp90 inhibition up-regulates protective Hsps (especially Hsp70) and potentially down-regulates NF-κB by disruption of the IkB kinase (IKK) complex. However, the clinical application of Hsp90 inhibitors is currently limited by their toxicity profile and the exact mechanism of protection conferred is unknown. Toll-like receptor 4 (TLR4) is a further regulator of NF-κB and recent studies suggest TLR4 plays a dominant role in mediating kidney damage following IRI. The full interaction of Hsps with TLRs is yet to be delineated and whether TLR4 signalling can be targeted by Hsp90 inhibition in IRI remains uncertain.

Expert opinion: Pharmacological pre-conditioning by Hsp90 inhibition involves direct treatment to the kidney donor and/or organ, which aims to reduce injury prior to the onset of ischemia. The major challenges going forward are to establish the exact mechanism of protection offered by these drugs and the investigation of less toxic analogues that could be safely translated into human studies.

Keywords: heat shock proteins, ischemia–reperfusion injury, kidney, transplantation

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1. Introduction

In 2009 the incidence rate of end-stage renal disease (ESRD) in the USA was 371 per million population [1], a figure that is rising rapidly and unlikely to reach steady state for another 25 years [2].

Kidney transplantation is the gold standard treatment for ESRD and is associated with significantly lower mortality and increased quality of life [3]. However, there is unfortunately an increasing disparity between numbers of patients active on the renal transplant list and the availability of donor organs [4].

Ischemia–reperfusion injury (IRI) is an unavoidable consequence of the transplantation procedure and is responsible for delayed graft function (DGF) in approximately 25% of kidneys obtained and transplanted from donors with brain stem death and up to 50% of kidneys from donors after cardiac death [5]. DGF is consistently associated with worse outcomes including a 38% increase in the relative risk of acute rejection and 41% increased risk of graft loss after 3 years of follow-up [6].

The concept of treating an organ in order to protect it, prior to a known impending injury is termed pre-conditioning and was first described in the heart by Murry et al. in 1986 [7]. Since then many different physical and pharmacological
pre-conditioning strategies have been explored in organ transplantation and in the context of renal IRI. One of the most recent strategies described involves the pharmacological induction of heat shock proteins (Hsps).

2. Heat shock response

The heat shock response was first discovered in 1962 in *Drosophila* by Ferrucio Ritossa [8] and encompasses the up-regulation of a group of highly evolutionarily conserved proteins, known as Hsps, that are well recognised for cytoprotective activity in a multitude of injury-repair states [9]. A wide range of stressors, including heat, infection, hydrostatic pressure, oxidative stress, chemical insults and ultraviolet radiation induce the heat shock response [10,11] and crucially this confers protection from subsequent insults [12].

Owing to the generality of this phenomenon, Hsps are often called stress proteins [13], however they have been described as “cellular mechanics” [14] and are commonly referred to as molecular chaperones. They have been found to play a fundamental role in the correct folding of newly synthesised proteins, the re-folding of proteins that are denatured by various stressors and post-translational translocation of proteins to their sites of action [15]. They are also capable of activating cell signalling pathways [16] and inhibiting caspase-dependent apoptosis [17].

Hsps, therefore, are strongly associated with cellular protection and up-regulation in the clinical setting promises significant benefits. Transplantation necessitates inflicting an ischemic injury on an organ, but the planned nature of this insult presents an opportunity to act to reduce the magnitude of this injury. There is currently no active pharmacological agent used at the time of organ donation to reduce IRI, therefore the use of an agent in this clinical setting would be unique.

3. Heat shock protein regulation

Heat shock transcription factor 1 (HSF1) is the main regulator of heat shock genes [18]. It is found normally in the cytoplasm in an inactive form, bound to a multi-chaperone complex that includes Hsp90 [19]. In order to achieve transcriptional activation, HSF1 must dissociate from this complex, form a homotrimer, move to the nucleus and become hyperphosphorylated [20]. Hsp90 is the primary regulator of HSF1 with binding of Hsp90 to HSF1 maintaining HSF1 in its inactive, compacted form in the cell. In the presence of cellular stress, there is an increased concentration of unfolded proteins [21]. These compete with the Hsp90 multi-chaperone complex allowing free HSF1 to dissociate and be transported to the nucleus where it binds to specific promoter regions with resulting Hsp transcription (Figure 1) [19].

4. Heat shock protein 90

Hsps are subdivided into several families according to their molecular mass [22] and occur in constitutive or inducible forms [23]. The Hsp90 molecular chaperone family is expressed in almost all organisms and under normal conditions Hsp90 accounts for 1 – 2% of all cellular proteins [24]. As a result, Hsp90 is one of the most plentiful cytoplasmic proteins in the unstressed cell [25]. When a cell is exposed to physiological stressors including heat, hypoxia, acidosis and heavy metals, basal levels can be increased up to 10 times [26,27].

Hsp90 is a ubiquitous homodimeric molecular chaperone [28] with two cytosolic isoforms termed α and β [29]. Dimerisation occurs when the isoforms form homodimers with the highly conserved C-terminal site of Hsp90 [25].

Hsp90 congregates with other molecular chaperones to form a large protein complex, known as a multi-chaperone complex. This chaperone complex has diverse functions including the prevention of aggregation of unfolded proteins under stressful conditions and regulation of the folding and maturation of a number of signal transduction molecules and receptors [30]. Hsp90 interacts with numerous client proteins in this complex including co-chaperones (such as Hsp70), signal transducers, activators of transcription and potentially IkB kinase (IKK) [31]. However, as an ATP-binding protein [25], Hsp90 is dependent on its intrinsic ATPase activity to be able to fulfil this chaperone purpose [32].

Being a member of the GHKL family of ATPases, Hsp90 exhibits a distinctive N-terminal nucleotide-binding pocket [33]. ATP hydrolysis and ADP/ATP nucleotide exchange in the N-terminal domain induce a conformational change of the protein [25] and are essential for Hsp90 chaperone function [28].

Evidence for a central role of Hsp90 in the regulation of HSF1 includes the observation that Hsp90 inhibitors can activate all steps of the stress protein response. Indeed,
activation of HSF1 occurs uniformly in response to all Hsp90 inhibitors currently under clinical evaluation [34]. In this sense, HSF1 is an unusual Hsp90 client protein: other Hsp90-associated proteins become destabilised and degraded by proteolysis on Hsp90 dissociation, while HSF1 is activated leading to Hsp induction [35].

5. Hsp90 inhibitors

The identification of geldanamycin marked the first description of a group of drugs termed the benzoquinone ansamycins. Geldanamycin was first isolated from streptomyces hygroscopicus in the early 1970s and was noted for its antiproteasome activity. Herbimycin A was subsequently identified and was the first of these compounds to be identified as an agent capable of short circuiting the Hsp response [36].

The anti-tumour potential of the benzoquinone ansamycins was suggested in the late 1970s but it was not until the mid-1980s that first herbimycin [37] and then geldanamycin [38] were shown to inhibit the malignant transformation of fibroblasts by the v-Src oncogene. Src kinase belongs to a family of non-receptor tyrosine kinases and the benzoquinone ansamycin effect was believed to be due to tyrosine kinase inhibition. It was, however, shown that Src formed a stable complex with Hsp90 and the benzoquinone ansamycins were specific Hsp90 inhibitors [39]. Hsp90 inhibition prevented complex formation resulting in Src degradation by the ubiquitin-proteasome pathway. Since this discovery various other oncogenic proteins including HER2, EGFR, mutant ER, HIF1α, Raf-1, AKT and mutant p53 have been identified as requiring functioning Hsp90 in order to exert their effects [40].

While geldanamycin, herbimycin and then radicicol were the first natural Hsp90 inhibitors discovered, their instability and hepatotoxicity limited any clinical potential [34]. The geldanamycin analogue 17-allylamino-17-demethoxygeldanamycin (17-AAG) was better tolerated and the first of these compounds to be assessed in a clinical trial. A side-chain modification of 17-AAG resulted in the water-soluble geldanamycin analogue, 17-demethylamino-ethylamino-17-demethoxygeldanamycin (17-DMAG). Since then numerous other smaller molecule Hsp90 inhibitors have been discovered representing some of the most actively pursued cancer drug treatments by the pharmaceutical industry, with 17 agents having entered clinical trials [41].

Although cancer treatment has formed the focus of research in Hsp90 inhibitors, therapeutic application has been demonstrated in a number of other disease states [19]. These include autoimmune encephalomyelitis [42], sepsis-induced lung injury [28], endotoxin-induced uveitis [43], rheumatoid arthritis [44] and atherosclerosis [31]. In addition, Hsp90 inhibitors are also capable of blocking the activity of pro-inflammatory mediators, inhibiting Hsp90-dependent immune pathways [45], attenuating oxidative stress [46], reducing viral replication [47] and treating fungal infection [48]. Moreover, Hsp90 inhibition has therapeutic potential in the treatment of neurodegenerative diseases including Alzheimer’s disease, Parkinson’s disease, Huntington’s disease and motor neurone disease – through prevention of the aggregation and toxicity of misfolded proteins such as amyloid β, tau, α-synuclein, mutant huntingtin and mutant superoxide dismutase-I [49].

Hsp90 inhibitors prevent nucleotide binding to Hsp90, resulting in client protein destabilisation, deactivation and degradation [50]. Why Hsp90 inhibition leads to cellular protection is unclear but is likely a consequence of two main effects. Firstly, HSF1 is released from the repressive multi-chaperone complex with subsequent up-regulation of Hsp expression (especially Hsp70) [51] and secondly by NF-κB deactivation by disruption of the IKK complex with Hsp90 (Figures 1 and 2) [31].

6. The up-regulation of anti-inflammatory proteins (Hsp70 in particular)

Hsp90 inhibitor blockade of the Hsp90 ATP-binding site leads to both degradation of client proteins via a ubiquitin--proteasome-dependent pathway and up-regulation of other Hsps, particularly Hsp70 [51]. The Hsp70 family is capable of protecting cells from lethal heat and other insults [52]. Since Hsp70 functions to prevent protein aggregation and facilitates the refolding of denatured proteins, up-regulating Hsp70 as a pre-conditioning strategy has been shown to be cytoprotective in a number of organs including the kidney and heart [53-59].

The exact mechanism by which Hsp70 reduces injury remains uncertain but it has been postulated that it may result from IκB stabilisation or prevention of NF-κB p65 translocation [60-64]. Another possibility is that Hsp70 leads to ubiquitinisation and proteasomal degradation of certain pro-inflammatory Hsp90 client proteins [28].

7. The inhibition of pro-inflammatory transcription factors (in particular NF-κB) and cytokines

IκB is an inhibitory protein that masks the nuclear localisation signal sequence of NF-κB [28]. Therefore, activation of NF-κB requires phosphorylation and dissociation from IκB by IκK [65]. IκK is composed of two protein kinase subunits IκKα, IκKβ as well as the regulatory subunit NEMO [65]. Hsp90 may be needed to stabilise the IκK complex [66] and the use of Hsp90 inhibitors could cause the dissociation of the IκK complex and prevention of NF-κB activation [67]. Indeed, geldanamycin has inhibited TNFα-mediated IκK and NF-κB activation in a number of in vitro models [68-72].

Pittet et al. demonstrated this effectively when observing that heat treatment or sodium arsenite stress induced dissociation of Hsp90 protein from the IκK complex rendering the IκK complex detergent insoluble. Simultaneously,
it was observed that the NF-κB system became impervious to cytokine stimulation [73].

This theory of protection is endorsed by Jo et al. who investigated the effect of heat pre-conditioning in a rat kidney IRI model. In this study, heat pre-conditioning protected the kidney, induced Hsp70 and suppressed IRI induced NF-κB activation. The administration of quercetin, a widely known but non-specific inhibitor of Hsp70, decreased Hsp70 expression markedly with associated loss of the functional protection from IRI conferred by heat pre-conditioning. Quercetin treatment was also associated with a reversal of NF-κB suppression [9].

Additionally, Ran et al. demonstrated Hsp70 binding to the coiled-coil domain of NEMO, which prevented the oligomerisation of NEMO and arrangement of the active IKK complex [63]. Weiss et al. observed that Hsp70 disturbed the role of IKK complex, impairing IκB phosphorylation and degradation and preventing translocation of NF-κB [64].

8. The role of Hsp90 in modulating inflammation in the kidney

Hsp90 are crucial regulators of the inflammatory response to ischemic injury in kidney. This is affirmed by Zhang et al. who used micro-array analysis to compare gene expression in rat kidneys subjected to IRI. Of the 30,000 genes analysed, Hsp70 gene product up-regulation was greatest (43-fold), followed by Hsp27 (12-fold) and hemeoxygenase-1 (10-fold) [74].

Increasing evidence supports a role for Hsps during the recovery from renal ischemia, especially in the tasks of cellular rescue from apoptotic cell death and cytoskeletal restoration [75]. Pioneering work by Perdrizet et al. demonstrated that total body hyperthermia, followed by recovery, caused enhanced Hsp72 production and protected renal allografts from cold and warm ischemia [76,77]. Since then Hsp induction has been shown to be associated with improved kidney viability and function post IRI in a variety of experimental models (Table 1) [9,56,77-91].
In the kidney, IRI induces inflammatory mediators such as adhesion molecules, cytokines and chemokines [92]. These mediators are thought to instigate an inflammatory cascade leading to leukocyte recruitment and microcirculatory compromise with ensuing renal dysfunction.

As many of these mediators have \( \kappa B \)-binding regions, their transcriptional regulation is thought to be under the control of NF-\( \kappa B \) [93,94]. Therefore, NF-\( \kappa B \), which is likely to be regulated by Hsp70 and 90 via the IKK complex [67], may be a potential therapeutic target in ischemic renal injury [9] and specifically through the use of Hsp90 inhibitors.

During an investigation of lung pre-conditioning, Pittet et al. showed that treatment of rats with two intraperitoneal doses of the Hsp90 inhibitor geldanamycin induced renal Hsp70 [95]. Our own research group also observed this effect and went on to show that use of Hsp90 inhibitors protected primary renal tubular epithelial cells from oxidative stress in vitro and conferred functional and morphological protection in a mouse model of kidney IRI [78].

9. The role of immunity and toll-like receptors

Toll-like receptors (TLRs) are a family of trans-membrane proteins, named after the Drosophila protein Toll with which they share structural homology [96]. TLRs are highly conserved germline-encoded pattern recognition receptor (PRRs) [97] that are central to immune responses [98] including NF-\( \kappa B \) regulation [99].

It is now appreciated that TLRs not only represent the major PRRs across species but that they also recognise specific endogenous damage-associated molecular pattern molecules (DAMPs) that have been transformed from their native state or gathered in non-physiological sites or abnormal amounts during tissue injury [100].

Ischemic injury is thought to lead to necrosis and apoptosis of renal tubular cells leading to production of DAMPs [98]. These are recognised by local TLRs and promote inflammation through the production of cytokines and chemokines, and enrolment of immune cells that propagate the initial injury [98].

TLRs may be expressed by kidney tubular epithelial cells and mesangial cells in response to insults such as IRI [101]. Kim et al. demonstrated that rat kidneys subjected to IRI showed enhanced TLR mRNA and protein expression [102] and there is growing experimental evidence emerging to suggest that engagement of TLRs by endogenous ligands may be a key trigger of inflammation following ischemia in the kidney [103].

10. The role of TLR4

As shown by in situ hybridisation, TLR4 is constitutively expressed at the RNA level on renal epithelium and this expression is enhanced upon IRI [104]. In mice subjected to renal ischemia, TLR4 expression is especially amplified at sites most susceptible to IRI, such as in the proximal renal tubules at the cortical–medullary junction [104]. This expression is independent of pathogen invasion and TLR4 appears to recognise endogenous molecules that are exposed during cellular injury and extracellular matrix remodelling [101]. To date, a number of proposed endogenous ligands for TLR2 and TLR4 have been found in ischemic renal tissue, including high mobility group box 1 (HMGB1) protein, hyaluronan, biglycan [103] and Hsp70 [102].

In vivo experiments have confirmed reduced organ injury in kidney IRI in transgenic mice missing TLR4 or the TLR4-related scaffolding protein MyD88 [105,106]. Results of
experiments by Pulskens et al. also show lower levels of chemokines and inflammatory cells, less renal injury and more preserved renal function following IRI in TLR4−/− mice as compared to wild types [105]. Wu et al. used chimeric mice to show that TLR4 expressed specifically on tubular epithelial is an important modulator of ischemic injury [103], while Chen et al. extended this role of TLR4 into the endothelium of the kidney and demonstrated that leukocytes release IL-6 when TLR4 receptors are stimulated by HMGB1 release from injured cells [107]. In a rat model, Liu et al. showed that inhibition of TLR4 by the drug Eritoran (a structural analogue of the lipid A portion of lipopolysaccharide) attenuated the signalling cascades in renal IRI [108].

Similarly, in the case of TLR2, Leemans et al. [109] and Shigeoka et al. [110] both demonstrated that parenchymal cell deficiency of this receptor dramatically limited kidney injury following IRI, and diminished the associated renal inflammation. However, in further studies Rusai et al. found that a double genetic deletion of TLR2 and TLR4 conferred only similar protection to single deletions of TLR2 or TLR4 [111].

This collective body of research suggests that TLR4 signalling plays a dominant role in mediating kidney damage following IRI [103,107], with at least three major cell types (epithelia, endothelia and leukocytes) being identified as expressing TLR4 at various times during ischemic kidney injury [107]. Moreover, it has been demonstrated that for the classical response to ischemia to occur there is an absolute necessity for TLR4 in these cells [103,107,112].

This experimental research also appears to translate to the clinical situation, as a recent study in humans confirmed the role of the donor TLR4 and HMGB1 interaction in kidney transplant IRI [113]. This is in keeping with previous suggestions that cadaveric kidneys may experience an increased ischemic injury in response to danger signals provided by the recipient’s innate immune system [114].

11. Hsp90 inhibitors and immune regulation

Although IRI typically occurs in a sterile environment, activation of innate and adaptive immune responses occurs and contributes to injury, including activation of PRRs such as TLRs [115]. A number of key signalling proteins that are Hsp90 clients play important roles in the cellular activation of both innate and adaptive immune responses. Therefore, a rationale exists for targeting Hsp90-dependent pathways in immune-mediated diseases [45].

In rats, 17-AAG has been shown to halt the development of lipopolysaccharide-induced autoimmune uveitis by inhibiting activation of multiple signalling molecules that mediate the TLR4-induced pro-inflammatory cytokine response in retinal cells [43].

Likewise, Dello Russo et al. demonstrated that 17-AAG blocked pro-inflammatory TLR4 activation in vitro and dramatically decreased disease incidence and severity in an experimental model of autoimmune encephalitis [42]. More recently, Yun et al. used a synthetic Hsp90 inhibitor, EC144, to prevent lipopolysaccharide-mediated TLR4 signalling in RAW 264.7 cells through inhibition of ERK1/2, MEK1/2, JNK, and p38 MAPK but not NF-κB [45].

The above evidence suggests that inhibiting Hsp90 function may be applicable to treatment of autoimmune diseases, but whether TLR4 signalling can be similarly targeted in IRI remains uncertain and to date the interaction between Hsp and TLRs (a controversial area in the literature) is yet to be fully delineated.

12. Interaction of Hsp and TLRs

Members of the heat shock protein family, including Hsp60, 70, 72, 90 and gp96, are capable of inducing production of pro-inflammatory cytokines via CD14/TLR2 and CD14/TLR4 receptor complex-mediated signal transduction pathways [116]. Ohashi et al. found that the pro-inflammatory signalling of human Hsp60 was found dependent on a functional TLR4 [117]; whereas Vavulas et al. defined Hsp70 as an endogenous stimulus for the Toll/IL-1 receptor signal pathway that engages TLR2 and TLR4 [118].

In an experimental study investigating cardiac IRI, it was shown that extracellular Hsp70 plays a role in IRI by TLR4-dependent mechanisms and that recombinant Hsp70 induced NF-κB activation as well as the expression of TNFα, IL1β and IL-6 [119]. In the context of renal IRI, Kim et al. suggested that enhanced TLR mRNA and protein expression in a rat model of IRI was due to endogenous TLR ligand levels of Hsp70 within tubular cells [102].

However, the mechanism by which Hsp produce cellular activation through TLR2- and TLR4-dependent mechanisms is not well understood, and there is continuing debate regarding the importance of interactions between Hsps and TLRs [106].

It has been argued that early investigations identifying Hsps as TLR ligands were the result of contamination with pathogenic ligands since the reported cytokine effects of Hsps in vitro were similar to pathogen-associated molecular pattern molecules (PAMPs) such as lipopolysaccharide from Gram-negative bacteria [96]. Since then a number of in vitro techniques have evolved to nullify the risk of lipopolysaccharide contamination including protein expression in eukaryotic hosts and chaperone treatment with polymyxin B, neutralising monoclonal antibodies and acyloxyacyl hydro-lase. Other techniques include the use of various controls including chaperone antibodies, mutants of the chaperone and lipopolysaccharide itself. If the latter is used, micro-array analysis is effective at determining differences in response. Finally, purity can also be tested with a limulus assay, mass spectrometry and SDS-PAGE [120].

There is also research that highlights other trans-membrane receptors such as CD91 and LOX-1 as the principle Hsp70 receptor. This evidence may call into question the importance of Hsp and TLR interaction. On the other hand, the implication of Hsp70 signalling through multiple germline-encoded
immune receptors may serve to further underline the potential importance of Hsp70 in immune regulation [121].

Either way in vivo Hsp and TLR interactions that have been well described cannot be explained by contamination. Therefore, another argument against Hsp and TLR interaction has evolved and suggests that molecules bound to, or chaperoned by, Hsp can activate TLRs, rather than the Hsp molecules themselves [122].

Despite these arguments, it has been shown that dying cells often undergo the stress protein response, leading to lysis and release of Hsp into the extracellular space [123]. This is thought to be able to activate not only inflammatory but also immune responses [124]. Conversely, Hsp60 has been reported as being translocated to the surface of apoptosing cells as a recognition element for phagocytotic clearance, thereby attenuating an inflammatory response [125]. In addition, HSF1 has been identified as a transcriptional repressor with a role in the counter-regulation of cytokine gene transcription [126]. As a result the pro-inflammatory effects of extracellular Hsp have been described as being countered by the intracellular stress protein response in what is now known as a dichotomy of effects, which is best depicted in the case of Hsp70 [124].

13. Extracellular Hsp70 and potential pro-inflammatory effects

Hsp70, like Hsp90, has immune regulation properties with both pro- and anti-inflammatory effects [127] and large clinical studies investigating cardiac ischemia have provided inconsistent reports of protective [128,129] or detrimental [130] effects of this chaperone.

Hsp70 residing in its cytosolic compartment appears to have the ability to decrease pro-inflammatory signalling cascades, thereby down-regulating inflammatory responses. In contrast, extracellular Hsp70 is thought to exhibit powerful immune-stimulatory effects and has therefore been acknowledged as a “chaperokine” [127].

The overall action of Hsp70 on the inflammatory equilibrium is reliant on the cellular compartment in which Hsp70 displays its predominant effect, which depends mainly on the extent of the tissue injury [121].

In general systemic stress, there is reliance on NF-κB, in addition to up-regulation of intracellular Hsp70 in inflammatory cells [131-133]. In this scenario, pro-inflammatory messages via innate immune receptors can be overcome by the intracellular down-regulating action of Hsp70 on NF-κB signalling, thereby reducing the undesirable effects of systemic inflammation [121]. On the contrary, the response could be pro-inflammatory when in the absence of cytosolic stress, there is a lack of intracellular Hsp70 up-regulation and absence of its down-regulatory effect on NF-κB [121].

This hypothesis is in agreement with the “danger theory” described by Matzinger [134], in which DAMPS such as Hsp are released from damaged cells providing the immune system with a “danger” signal. The response to that hazard is initiated by antigen-presenting cells that identify Hsp via TLRs resulting in up-regulation of NF-kB and activation of pro-inflammatory cytokines [135].

In summary, the novel functions of Hsp70 could depend on its location with intracellular Hsp70 attenuating inflammatory cascades, while Hsp70 released into the extracellular space specifically binds to TLR2 and 4 and produces immune-regulatory effects, including up-regulation of adhesion molecules, co-stimulatory molecule expression, and cytokine and chemokine release [124]. It is predicted that in Hsp90 inhibition intracellular up-regulation of Hsp70 predominates and exerts a protective effect through an as yet unknown mechanism. However, this theory requires confirmation and is a focus of ongoing research. A recent review has highlighted that studies aimed at more accurately characterising the molecular pathways activated by Hsp70 are required to determine its overall effect [136].

14. Routes of administration and dosing regimens

In the pursuit of Hsp90 inhibitors that are safe for clinical use, a major limitation of 17-AAG is its poor solubility in water. To improve solubility, DMSO in egg phospholipid and cremophor-based formulations were initially developed but required anti-allergic premedication as well as specialised giving sets for intravenous administration. To overcome these problems, injectable isotonic suspensions, oil-in-water nano-emulsions and newer albumin-bound forms of 17-AAG have emerged to improve solubility and deliver higher concentrations of drug to target sites. 17-DMAG being water-soluble is easier to administer and can be given both intravenously and orally [41].

In a recent Phase II study investigating 17-AAG, 11 patients with metastatic or locally advanced irresectable breast cancer were given dosing regimens of 220 mg/m² IV over 2 h on 5 separate days during a 21-day chemotherapy cycle. Five of the study group developed grade 3/4 toxicities, which were primarily hepatic and pulmonary [137]. In Phase I studies of 17-DMAG in patients with advanced solid tumours, the recommended doses for future Phase II investigations were found to be 16 mg/m² × 5 days and 25 mg/m² × 3 days every 3 weeks administered by hour long intravenous infusion. Similar to 17-AAG, the most common grade 3/4 toxicities encountered with 17-DMAG were hepatic (14%) and pulmonary (9%) [138]. The doses that lead to these significant side effects are high and often at the maximum tolerated level. However, the dose required to give cellular protection is predicted lower and thus associated with less toxicity. For example, doses of 1 mg/kg of geldanamycin, 17-AAG and 17-DMAG have previously conferred renal protection in a mouse model of IRI [78].

15. Toxicity profile of Hsp90 inhibitors

Hsp90 is exploited by cancer cells to sustain oncoproteins including many mutated kinases and transcription factors...
that are overexpressed in cancer. Hsp90 also buffers cellular stress induced by malignant cells aiding their survival and propagation [34]. Use of Hsp90 inhibitors in the treatment of malignancy therefore does not lead to cancer cell death by cytotoxicity but by targeting and inhibiting these disease-dependent mechanisms.

Newer Hsp90 inhibition agents, some of which have good oral bioavailability, lack the hepatotoxic profile of first-generation Hsp90 inhibitors possibly due to an altered quinone moiety and rate of superoxide formation [119]. This allows for repeated administration at higher concentrations. However, the Hsp90 chaperone function extends to maintaining normal cellular homeostasis and concerns exist regarding the inhibition of specific Hsp90-dependent cellular mechanisms, at least on a long-term basis, which could be the case with certain chemotherapy regimens [34].

Since the treatment philosophy in organ pre-conditioning is different to that of cancer therapy, the implications of blocking normal homeostatic functions of Hsp90 are less important when considered in relation to IRI reduction. In this context, a Hsp90 inhibitor is administered on a once only basis either to the donor or directly to the kidney at a dose sufficient to up-regulate Hsp70 expression. Our group have previously seen a two- to threefold increase in Hsp70 in mice given 1 mg/kg of geldanamycin, 17-AAG and 17-DMAG into the peritoneum. Hsp70 expression was maximal at 6 h in mice treated with 17-DMAG, which was significantly earlier than in geldanamycin (8 h) or 17-AAG (16 h) treated groups, perhaps reflecting improved bioavailability as a result of water solubility. No toxic effects were observed over a 2-week period following treatment [78].

16. Isoform-selective Hsp90 inhibitors

In addition to the two cytosolic isoforms of Hsp90 termed α and β, two other human isoforms of Hsp90 exist: tumour necrosis factor receptor-associated protein (Trp-1), which is localised to the mitochondria, and glucose-regulated protein, Grp94, which resides in the endoplasmic reticulum. Recently, it has been suggested that the side-effects of Hsp90 inhibitors like geldanamycin, 17-AAG and 17-DMAG may be due to pan-inhibition of Hsp90 and that development of isoform-selective may be a means of reducing toxicity. Interestingly there is also known to be a number of pro-inflammatory mediators that are Grp94-dependent clients, including TLR-4 [140]. This opens up the possibility that Hsp90 inhibitors may exert a protective effect through Grp94 inhibition.

17. Conclusion

Pharmacological induction of Hsp expression is an emerging pre-conditioning strategy aimed at reducing IRI following organ transplantation and other ischemic injuries. Hsp90 inhibitors lead to up-regulation of Hsp expression (especially Hsp70) and potentially block NF-κB activation by disruption of the IKK complex. The importance of TLR4 signalling in mediating kidney damage following IRI is now clear. Hsp90 inhibition is an attractive strategy to reduce TLR4-mediated IRI but as yet remains unproven.

18. Expert opinion

Despite great improvements in outcome following kidney transplantation, the incidence of graft failure remains high. The shortage of organs for transplantation has also led researchers to look for new techniques to expand the donor pool resulting in the increasing use of grafts from expanded criteria and donation after cardiac death donors. These kidneys suffer greater ischemic injury leading to worse outcome.

Developing a pharmacological strategy to reduce IRI, the main contributor to early graft failure is highly desirable. The ultimate aim is to improve the function and longevity of transplanted organs by administering a single dose of an agent to an organ donor prior to organ retrieval. This would prime protective cellular mechanisms in anticipation of the injurious nature of ischemia and reperfusion. In addition, it may be beneficial to treat the organ ex vivo with a drug delivered by machine perfusion prior to the implant procedure.

There is currently no therapeutic means of treating kidneys destined for transplantation in order to improve post-transplantation function, a process termed pre-conditioning. The use of an agent in this clinical setting would therefore be unique. Pre-conditioning strategies also have the potential to improve the function of substandard organs so that they become suitable for transplantation, thus expanding the donor pool.

Donor pre-treatment clearly has much potential because it would allow prevention and treatment of IRI at the earliest stage. However, there are ethical, logistical and organ-specific issues that need resolved before these strategies can be implemented in a clinical environment. These issues revolve around legislation, consent of donor/recipient and the fear that some pre-conditioning agents may be beneficial to one organ but not to others.

Although a number of alternative pre-conditioning drugs have shown promise in preclinical trials, clinical efficacy has proven harder to demonstrate in Phase I/II trials [115]. Humanised IgG4 monoclonal antibody to human TLR2 (Opsona) and Diannexin (Astellas) are currently in Phase I/II trials, but no information on efficacy has been made public yet. The pre-conditioning strategy described in this review involves direct treatment to the organ donor and/or organ, aiming to reduce injury prior to the onset of ischemia. Other strategies (including anti-TLR2 and Diannexin above) are aimed at treating the organ recipient, which may be too late.

Further debate surrounds the potential benefit of pharmacological strategies over physical pre-conditioning manoeuvres. In the latter, cellular protection is achieved by repeated temporary interruption of blood flow to an organ or distant site. A pilot study has recently demonstrated a reduction in acute kidney injury after heart surgery following remote
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pre-conditioning of the leg [141]. However in transplantation, organ physical pre-conditioning has been studied almost exclusively in liver and on the whole, results have been disappointing [142]. Pharmacological pre-conditioning also has advantages over a physical pre-conditioning strategy as an ischemic injury with associated negative consequences is avoided and time-consuming, surgeon-dependent intraoperative manoeuvres are not required.

To date using the older Hsp90 inhibitors, geldanamycin and related analogues (17-AAG and 17-DMAG), we have demonstrated up-regulation of Hsps together with protection against oxidative injury (in cell culture) and IRI in mice [78]. Hsp90 inhibition has additionally been shown to induce Hsp70 up-regulation in the liver, lung and heart of mice [78]. Hsp90 inhibitors therefore have potential benefits for all organs retrieved in the multi-organ donor setting. These donors carry huge socioeconomic value and improving early function and longevity of transplanted organs in this setting is a high priority area. This could represent a particular advantage of Hsp90 inhibitors over other agents.

However, renal IRI is not unique to transplant surgery and occurs in variety of different clinical settings including shock, sepsis, exposure to nephrotoxic medications, vascular surgery and cardiothoracic surgery. This leads to an acute ischemic kidney injury, which not only causes potentially life-threatening renal failure but also ignites a pro-inflammatory cascade through the release of inflammatory mediators that lead to a systemic response eventually resulting in remote organ injury and multi-organ failure.

Defining a method of inducing protective Hsp expression in transplantation could lead to a reduction of renal IRI in these other allied surgical disciplines as well as opening up avenues of investigation for treatment of non-surgical causes of acute ischemic kidney injury. Furthermore, the findings could be extrapolated to ischemic injury in other organs, such as the brain and heart.

Administering the currently available Hsp90 inhibitors at a lower once only dose is unlikely to significantly block the normal homeostatic functions of Hsp90 or lead to the level of toxicity observed in chemotherapy regimens. However, pre-conditioning agents should ideally not have any harmful side effects, particularly in a living donor and donation after cardiac death setting when the donor is still alive. Therefore, the major challenge going forward is to develop less toxic Hsp90 inhibitors that could be safely utilised in human subjects. The exact mechanism of protection is also unknown and is the matter of current investigation.

**Declaration of interest**

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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