Manipulating the HIF Pathway in Renal Transplantation, Current Progress and Future Developments

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Abstract

Renal transplantation is the gold standard treatment for end stage renal disease; however, there are currently not enough suitable organs to meet a growing demand. The Hypoxia Inducible Factor (HIF) pathway comprises a number of oxygen sensitive transcription factors which activate cellular protective functions. Allografts from cadaveric organs make up a large proportion of the organ pool. These organs are subjected to hypoxic and ischemic conditions during the harvesting and transplant process. Manipulation of the HIF pathway may reduce organ damage and improve outcomes. This review evaluates the current evidence for the use of the HIF pathway in renal transplantation and also adds new research conducted into the effect on organ metabolism.

Keywords: Transplantation; HIF; Renal; Metabolism

Introduction

The success of renal transplantation and ever pressing demand for donor organs has resulted in the utilisation of organs previously deemed to be non-transplantable including those termed 'marginal' and Extended Criteria Donor (ECD) kidneys [1-3]. These organs have worse associated clinical outcomes compared with living donor or standard criteria donor kidneys [1,2,4]. Given the global decline in cadaveric organ quality, the need to optimise their function is self-evident in order to strive for optimal patient outcomes.

Hypothermic Machine Perfusion (HMP) is a common method of organ preservation which involves the recirculation of cooled perfusion fluid through the renal vasculature. The clinical benefits of HMP are now well documented with reduction in Delayed Graft Function (DGF) and improved graft survival at 1 and 3 years [5,6]. In addition to these benefits, HMP provides a window during which novel therapies can be introduced into the perfusion fluid and delivered to cells within the renal parenchyma.

Organ ischemia results from cessation of blood flow following organ retrieval from both live and cadaveric donors. The resultant shift towards anaerobic metabolism is reflected in rising lactate levels as an end product of anaerobic respiration [7-9]. Hypothermia reduces cellular metabolism and oxygen requirement, along with a reduction in the rate of substrate and energy depletion. There is a 1.5-2 fold reduction in metabolism for every $10°C$ drop in temperature with activity present even at $1°C$. A consequence of anaerobic metabolism and the inefficient generation of ATP per glucose molecule is a rapid depletion of intracellular energy stores [10]. The lack of intracellular ATP leads to the failure of the ATP dependent sodium-potassium membrane pump, resulting in cellular influx of sodium and efflux of potassium with a loss of cellular integrity and ultimately, cell death. Thus rapid organ cooling following organ retrieval is essential in order to reduce short and long-term detrimental effects of prolonged anaerobic metabolism.

1D 1H Nuclear Magnetic Resonance (NMR) analysis of metabolites present in HMP perfusate has shown that metabolism occurs while under HMP conditions [7,11,12]. Moreover, the metobolomic profile of perfusion fluid appears to differ between kidneys with immediate and delayed graft function. Modification of metabolic processes such as the upregulation of the HIF pathway may improve outcomes for HMP kidneys.

The Hypoxia Inducible Factor (HIF) pathway is activated as part of the cellular response to hypoxia and exerts its beneficial effects via multiple mechanisms. Upregulation of the HIF pathway has been shown in an animal model to reduce Ischaemia Reperfusion Injury (IRI) [13-15] which is known to be deleterious during renal transplantation and is associated with an increased risk of DGF and reduced graft survival [15-17]. Furthermore, in hypoxic conditions, activation of the HIF pathway is associated with upregulation of mechanisms that facilitate glycolysis such as facultative glucose transporter-1, aldolase A, phosphoglycerate kinase 1 and pyruvate kinase M [18].

The increased delivery of glucose to the intracellular environment and subsequent glycolysis to produce ATP [19,20] would be seemingly beneficial during HMP given the glucose rich environment of commonly used perfusion fluids (Kidney Perfusion Solution 1/ University of Wisconsin Machine Perfusion Solution) in the absence of oxygen carriers or exogenous oxygen.

Aim

Given these two potentially protective mechanisms of HIF activation during organ preservation, namely amelioration of the IRI phenomenon and promotion of glycolytic activity, we aim to review and add to existing research on the role of the HIF pathway activation in renal transplantation and explore its manipulation in order to improve clinical outcomes following renal transplantation.

The HIF pathway and metabolic actions

The HIF pathway, described as the 'master regulator of the cellular hypoxic response, [21,22] has been shown to play key regulatory

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mechanisms in multiple disease states including acute ischemic renal failure and renal fibrosis [23,24]. HIF is a hetero-dimeric transcription factor composed of an oxygen dependent α subunit (1α,2α,3α) and an oxygen independent β sub-unit [24,25]. In normoxic environments HIF is rendered transcriptionally inactive. The α sub-unit is degraded by Propyl-Hydroxylase Enzymes (PHD-1, PHD-2, PHD-3), which require iron as a co-factor prior to subsequent ubiquitination and break down via von Hippel Lindau protein (pVHL) ubiquitin ligase pathway [24-31]. PHD inhibition up-regulates the HIF pathway by preventing this homeostatic mechanism. The von Hippel Lindau protein plays a key role in the degradation of the HIF pathway under normoxic conditions and the prevention of its over-expression (Figure 1) [31,32].

Experimental studies have highlighted distinct roles for the HIF sub-units in the hypoxic cellular response with HIF-1α activation demonstrating clear metabolic effects with the a shift in cellular metabolism from aerobic to anaerobic [25,32-34]. HIF-1α activation facilitates ATP production as a result of an increase in anaerobic glycolytic activity via the upregulation of both glycolytic pathway enzymes and glucose transporters (Glut-1) [34-36]. Further metabolic sequelae of HIF-1α activation is the inhibition of the TCA cycle via upregulation of pyruvate dehydrogenase kinase 1, which reduces the amount of the TCA substrate Acetyl-CoA [37].

Further beneficial effects of HIF-1α pathway activation are the improved respiratory efficiency in hypoxic cells [33,38,39]. This mechanism occurs via the regulation of cytochrome c oxidase (complex IV) as it catalyses the final step of the electron transport chain, the reduction of oxygen to water, thus increasing mitochondrial ATP generation [38-40]. In contrast, stimulation of HIF-2α has wide ranging effects including pro survival gene upregulation such as VEGF, TGF-a and EPO [41].

Manipulation of the HIF pathway

Mechanical and pharmacological upregulation of the HIF pathway has been described in animal transplant models. Mechanical upregulation of the pathway has been induced in a rat mode by bilateral clamping of the renal pedicle for 45 min [13]. This induces total ischaemia for the designated period, however, preconditioning leading to a hypoxic/ischaemic tolerance can achieved by sequential clamping and unclamping of the renal pedicle although the clinical application pre-retrieval of organs is understandably limited [42].
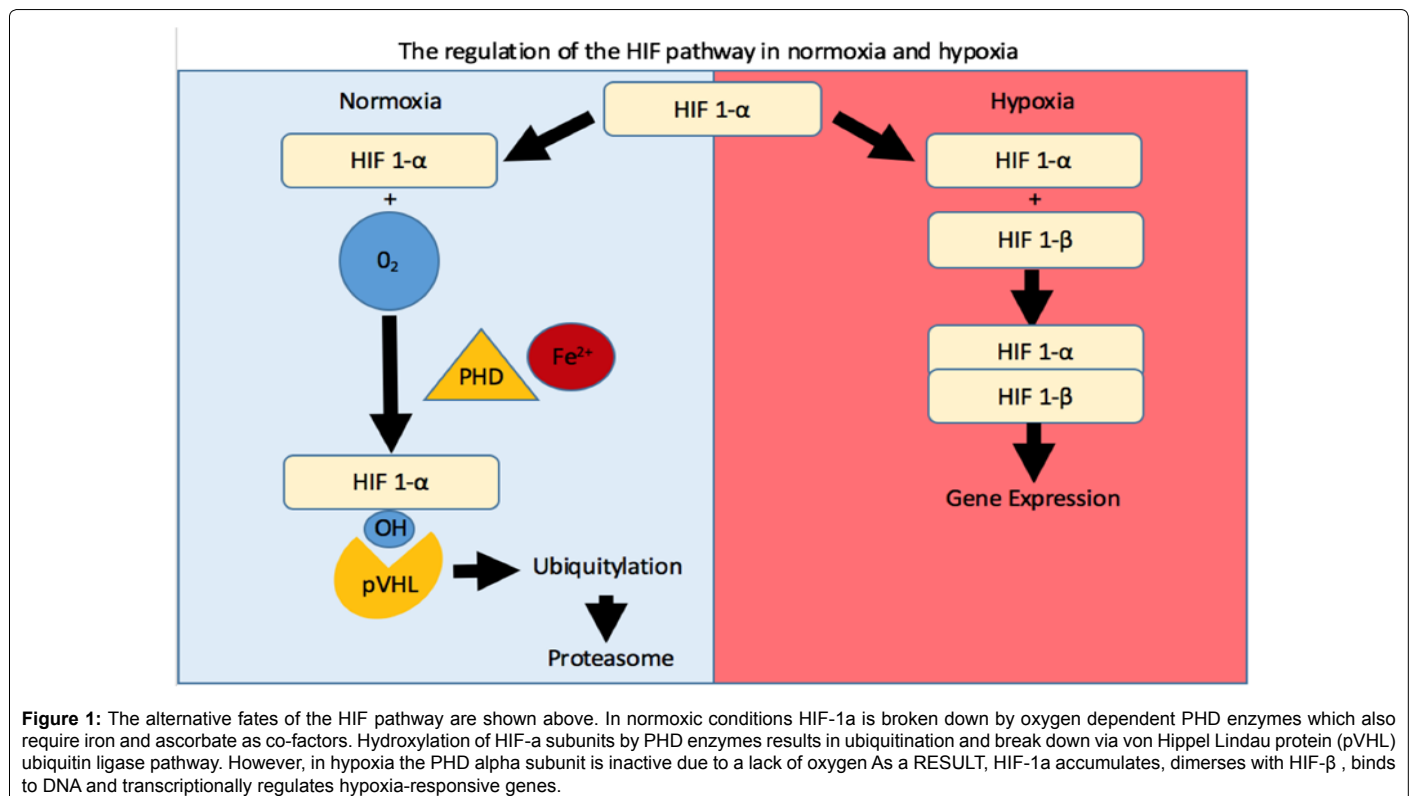
Pharmacological methods for inducing the HIF pathway include specific PHD inhibitors FG-4497 [43] or non-specific inhibitors such as desferrioxamine [44], xenon [45], fenoldopam [46] and dimethyloxalylglycine (DMOG) [47]. Interestingly, lactobionate which is a constituent of the University of Wisconsin (UW) preservation solution, is an iron chelator and therefore inhibits HIF via a mechanism similar to desferrioxamine, [48] as PHD enzymes depend on iron as a cofactor [15,49,50].

HIF in human observational studies

HIF-1α activation is one mechanism by which cells are protected from the effects of hypoxia, [34,51] thus, attempts to characterise and manipulate this pathway have been explored in both human [13] and animal studies [43,52].

Post-transplant HIF-1α levels have been shown to be upregulated in functioning grafts (CIT>15 h), and at considerably lower levels in non-functioning kidneys. The authors describe the trend that kidneys with increased post-transplant HIF-1α expression had lower rates of rejection, although this relationship was not statistically significant [53].

HIF-1α accumulates as a result of both renal ischaemia and also reperfusion in human proximal tubule cells, even after prolonged

Figure 1: The alternative fates of the HIF pathway are shown above. In normoxic conditions HIF-1a is broken down by oxygen dependent PHD enzymes which also require iron and ascorbate as co-factors. Hydroxylation of HIF-a subunits by PHD enzymes results in ubiquitination and break down via von Hippel Lindau protein (pVHL) ubiquitin ligase pathway. However, in hypoxia the PHD alpha subunit is inactive due to a lack of oxygen As a RESULT, HIF-1a accumulates, dimerses with HIF-β , binds to DNA and transcriptionally regulates hypoxia-responsive genes.

ischaemia, illustrating how both 'hits' are important [14]. Analysis of the biopsies of transplanted kidneys in the same study demonstrated HIF-1α was absent in kidney biopsies with marked ischemic damage and higher in proximal tubule cells with a lesser severity of ATN post-transplantation; expression was even higher in those cells with non-damaged tubule cells illustrating how HIF-1α stabilized during reperfusion may be required for proximal tubule survival and repair [13]. Accounting for this is the observation that proximal tubule cells have the potential to fully recover from ischemic pathology [54].

Rather than relying on a separate progenitor lineage, each individual Renal Proximal Tubule Epithelial Cell (RPTEC) has the potential to mediate tubule repair through an interplay between proliferation and efferocytosis of damaged cells [55]. *In vitro* studies have demonstrated that post ischemic insult, RPTEC de-differentiate and proliferate [56], undergoing a glycolytic switch to fuel expansion [57], rather than utilising fatty acids, the preferred metabolic entry point preferred by the mature PT epithelium [58]. This glycolytic switch coincides with increased HIF1 activity [57], which as described before upregulates genes necessary for glycolysis, suggesting a key role for HIF-1α in tubule repair.

The absence of HIF-1α from severely damaged tubules is also interesting, and suggests limited proliferation of the PTEC and therefore a finite reparative capability of the proximal tubule. Post-transplant proximal tubule function, in the absence of any other detectable underlying pathologies is linked with patient outcomes [59].

Parallel to this, HIF-1α has previously been thought to have a role in the late rejection of renal transplants by facilitating the process of renal fibrosis. The expression of HIF-1α was shown to be higher amongst infiltrating inflammatory cells in areas of tubular atrophy/ interstitial fibrosis in biopsies of chronic graft dysfunction, suggesting that the HIF pathway activation is associated with renal fibrosis [60].

Inactivation of the HIF-1α response via the use of HIF-1α small interfering RNA (siRNA), provoked significant cell death in a human proximal epithelial cell line further exposing the role HIF-1α plays in mediating proximal tubular cell survival in response to ischemia reperfusion injury both *in vitro* and *in vivo* [13].

The correlation between HIF-1α expression in graft biopsies and histological/clinical outcome is widely debated yet patterns have emerged. HIF-1α expression was shown to be higher in primarily functioning kidneys compared to those with primary non-function. Protocol biopsies at 2 weeks showed widespread HIF-1α expression with biopsies beyond 3 month void of HIF-1α other than in clinical/ sub-clinical rejection [53] thus implicating hypoxia in the process of chronic rejection. The presence of HIF-1α was absent in kidney biopsies with marked ischemic damage and only present in those with less severe injury leading to the hypothesis that the HIF pathway was responsible for protective mechanisms. Manipulation of the HIF pathway *in vivo* and *ex vivo* transplant models has been explored, however, no clinical trials have attempted to stimulate the HIF pathway in donors, recipients or during preservation of solid organ transplants [61].

Opportunities for the HIF pathway manipulation during the transplantation process

Manipulating the HIF pathway response in animal models: A transplant model using porcine kidneys demonstrated the increased expression of HIF-1α following transplantation and its potential positive role in tubular repair [62]. Interrogation of the HIF pathway response has been reported in several murine kidney models [13,45,52,60,63-66].

In a murine model those treated with Xenon or FG-4497 (Non-specific and specific HIF pathway inducers, respectively) prior to nephrectomy led to significantly improved results following transplant. The recipients of kidney transplants from those pre-treated animals demonstrated improved function (reduced DGF) following transplantation, which correlated with degree of HIF-1α up regulation [43,67]. Similar findings were demonstrated in a renal ischaemia model and the benefits of the HIF induction effects were abrogated with the introduction of HIF-1α siRNA [13,45]. The beneficial effects of pharmacological HIF-1 pathway stimulation appear to be long lasting with genetic (upregulation of EPO and HO-1) and functional effects of donor treatment persisting in the recipient post-transplant, even when these organs were subjected to significant period of cold ischaemia [65]. These results imply that in animal models, donor pre-treatment with a HIF pathway inducer has beneficial transplant outcomes and that activation of HIF is maintained under organ preservation conditions. Similar results have been reproduced when PHD inhibitors have been given to recipient mice following transplant with improved function demonstrated several weeks later [63].
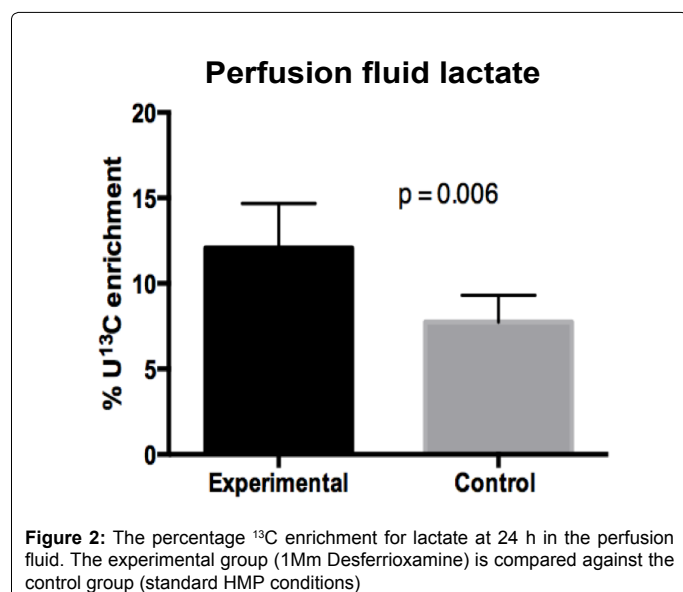
HIF pathway modification during HMP

Findings from animal studies indicate that the HIF pathway activation is not exhausted even after total mechanical ischaemia (such as during DCD retrieval conditions) [13]. Given that there are potential benefits to further HIF stimulation following organ retrieval and HMP can deliver pharmaceutical agents to the cells within an organ, it is perhaps surprising that there are no previous reports detailing the role of pharmacological HIF pathways stimulators during HMP.

Having previously used metobolomic analysis to show the porcine kidney behaves similarly to human kidneys [7] and that isotopic glucose 13C 2D-NMR tracer experiments can be used to determine new metabolism during HMP [8] we aimed to investigate the metabolic effects of pharmacological the HIF pathway activation while under HMP. We hypothesised that even after being subject to extreme ischaemic conditions in this DCD model, the HIF pathway could be further stimulated to alter the metabolism during HMP to facilitate greater anaerobic glycolytic activity.

Kidneys (n=6) were perfused for 24 h under HMP conditions following organ retrieval (Warm ischemia time: WIT 14 min). One kidney from each pair was perfused with fluid containing the non-specific PHD inhibitor (and therefore HIF inducer) desferrioxamine (1 mM) with the contralateral kidneys undergoing standard HMP serving as the control group. The perfusion fluid was similar to KPS-1 perfusion fluid with the substitution of glucose with the uniformly labelled non-radioactive isotope (U13C glucose) to allow metabolic tracer analysis.

The presence of fully labelled lactate (i.e., 13C in each carbon position) in the perfusion fluid demonstrates de novo glycolytic activity within the kidney with subsequent export from the perfused organ. The proportion of labelled lactate in the fluid after 24 h was found to be significantly higher in the presence of the PHD inhibitor (desferrioxamine) indicating upregulation of glycolysis (Mean 12.0% vs. 7.7% p=0.006) (Figure 2). Similarly, the proportion of labelled lactate was greater in the kidney tissue for the desferrioxamine treated group (14.0% vs. 9.3% p=0.008). Whilst the metabolic differences between the two groups may be due to the direct metabolic influence of the iron chelator desferrioxamine on the kidney, there was no difference in proportion of labelled lactate in the system after 6 h indicating that the impact on the metabolism of desferrioxamine is more protracted. Metabolic influence resulting from HIF activity would occur at a gene

Figure 2: The percentage ^{13}C enrichment for lactate at 24 h in the perfusion fluid. The experimental group (1Mm Desferrioxamine) is compared against the control group (standard HMP conditions)

transcriptional level and therefore the delay in metabolic impact of desferrioxamine in this kidney perfusion model would support HIF as the likely aetiology.

Although, the HIF pathway activation has been previously suggested as a being potentially beneficial in transplantation [61], to our knowledge this is the first report whereby the metabolic effects of HIF stimulation has been investigated during the HMP period. Although the functional consequences of HIF stimulation are not investigated in this study, we have shown in this animal model that the metabolic phenotype during HMP can be altered by the introduction of the iron chelator and non-specific pharmacological HIF pathway stimulator desferrioxamine. This finding corroborates previous studies, suggesting that in this DCD model, the HIF pathway is not exhausted despite periods of prolonged ischaemia [13]. Furthermore, the increase in the glycolytic endpoint U13C lactate in the desferrioxamine treated group demonstrates that metabolic upregulation of glycolytic activity is achievable in these *ex vivo* hypothermic hypoxic conditions which could potentially be used to optimise organ metabolism in the pre-transplant period.

Harmful effects of HIF pathway manipulation

Despite the potential benefits, there are also concerns regarding the possible harmful effects of manipulation of the HIF pathway in humans. There is some evidence in cancer studies that overexpression of the HIF pathway may have a role in hypoxia induced apoptosis [68]. VHL is a key component in the normoxic regulation and degradation of the HIF pathway, subsequently VHL mutation, specifically inactivation, plays a key role in clear cell renal carcinoma due to a loss of this feedback pathway [69]. This failure to break down HIF-1α has been shown to lead to overexpression of the HIF pathway which can predispose the cell to an invasive, highly vascular and cytotoxic resistant phenotype [70]. A link has also been demonstrated between high levels of the HIF pathway activation and renal fibrosis [71,72]. Although in other studies, correlating Banff scores from post-transplant histological samples with HIF-1α expression has not shown any significant relationship [73].

In addition, there are some concerns that HIF pathway stimulation can lead to immune mediated complications such as BK virus. HIF-1α has been known to interact with viruses with an increased HIF-1α expression reported in transplant biopsies from patients with active polyoma (BK) virus [74], a major cause of morbidity post transplantation [75].

Discussion

The HIF pathway plays a key role in the protective cellular response to ischaemia and hypoxia [13,47,70]. This has further been shown in transplantation models to have the potential to improve outcomes when active in donors prior to transplantation [43,45,46].

The murine studies discussed highlight the potential benefit of donor preconditioning via HIF pathway stimulation prior to organ retrieval [13,14,43]. However, these potential benefits remain at the experimental phase and have not been validated in the human setting. HIF pathway stimulation would be seemingly most advantageous for Deceased Cardiac Death (DCD) donors, who have an inevitable period of warm ischaemia. There are ethical concerns regarding the administration of 'organ-priming' drugs prior to organ retrieval [76] from which these donors who are alive at the time of drug administration would receive no direct benefit. Over activity of the HIF pathway is associated with several potential harmful effects [68,72,77] thus its modification should be approached with caution.

HMP provides a novel opportunity to pre-condition organs prior to transplantation. To our knowledge this is the first report to suggest that pharmacological stimulation of the HIF pathway during HMP can alter metabolism during the hypothermic hypoxic conditions of organ preservation. The metabolic shift to a more glycolytic metabolic state could perhaps be a demonstration of improved respiratory efficacy induced by HIF pathway upregulation. However, further research should increase the sample size and correlate changes in the metabolomics profile with clinical outcomes in order to explore the potential for pre-conditioning in the HMP setting.

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