Xenon preconditioning protects against renal ischemic-reperfusion injury via HIF-1α activation

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Abstract
The mortality rate from acute kidney injury following cardiovascular surgical procedures (such as thoraco-abdominal aortic aneurysm repair) can be as high as ~60% with no proven preventative therapy. Here we showed that preconditioning with the anesthetic gas xenon activates hypoxia-inducible factor-1α and its downstream effectors erythropoietin and vascular endothelial growth factor in a time-dependent manner in the kidneys of adult mice. Xenon-induced hypoxia-inducible factor-1α expression is due to its enhancement of translational efficiency via the mammalian target of rapamycin. Xenon preconditioning provided morphologic and functional renoprotection when given prior to kidney ischemia-reperfusion injury. The renoprotective properties of xenon preconditioning were abolished by hydrodynamic injection of small interfering RNA of hypoxia-inducible factor-1α. These results show that xenon preconditioning is a natural hypoxia-inducible factor-1α inducer and that exposure prior to renal ischemic insult can prevent acute renal failure. If these data can be extrapolated to the clinical setting, preconditioning with xenon would be beneficial prior to procedures in which renal perfusion is interrupted.
Acute kidney injury (AKI) due to ischemia followed by reperfusion is a significant clinical problem in cardiovascular surgical procedures such as thoraco-abdominal aortic aneurysm repair and renal transplantation. In these settings, interruption of renal blood flow is an inevitable component of the surgical procedure. The incidence of acute renal failure can be as high as 40% after aortic repair surgery and mortality is ~60% in patients who require hemodialysis.1, 2 The incidence of delayed renal graft function ranges from 2 to 50% worldwide from cadaveric donors3 and is 4 to 10% from living donors.4 Thus, renal ischemia-reperfusion injury is a common surgical problem which results in significant morbidity and mortality.

Preconditioning refers to protection of an organ from ischemic injury by a prior stimulus, and has been reported for many tissues including the kidney.5 The most extensively studied preconditioning stimulus is brief sub-injurious ischemia, which induces protection against subsequent ischemic injury. Preconditioning can also be triggered by pharmacologic agents including anesthetics. Although a number of different pathways have been implicated in preconditioning,6 the precise mechanisms underlying the protective effects remain incompletely defined; recently, interest has focused on involvement of hypoxia inducible factor-1α (HIF-1α) in mediating preconditioning.7

The noble gas xenon, discovered more than a century ago, has been used as an anesthetic since the 1950s albeit with a remarkable safety profile.8 Although sparingly used because of its cost, the utility of xenon preconditioning may come to the fore as an organ protective agent. Accordingly, the organ protective properties of xenon preconditioning have recently been demonstrated for the heart,9 and brain.10 Here, we
show that xenon preconditioning protects against ischemic reperfusion injury to the kidney in mice and that the mechanism is due to increased expression of HIF-1α as well as its downstream effectors, through enhanced translational efficiency involving the mammalian target of rapamycin (mTOR) pathway.

Results

Xenon preconditioning induces HIF-1α and its down stream effector expression.

To determine whether xenon preconditioning activates HIF-1α, we measured HIF-1α protein expression, and selected downstream effectors, erythropoietin (EPO) and vascular endothelial growth factor (VEGF), in the adult kidney following exposure of mice (C57BL/6J) to 70% xenon/30% oxygen for 2 hr. Kidneys from control mice (exposed to 70% nitrogen/30% oxygen) (control) had barely detectable levels whereas HIF-1α levels were significantly increased from 2 hr after xenon preconditioning (1.91 ± 0.5 vs 1.0 ± 0.02 of control, p < 0.05), with further up-regulation (3.86 ± 1.15 vs 1.0 ± 0.02 of control, p < 0.01) at 24 hr (Figure 1A). HIF-1α’s downstream effectors EPO and VEGF were also significantly increased by 160% and 110% respectively (p < 0.01) at 24 hr after xenon preconditioning compared to controls (Figure 1B,C). This is not an effect of anesthesia per se, since another anesthetic gas mixture, 70% nitrous oxide/30% oxygen, did not induce changes in HIF-1α expression (Figure 1D) or its downstream effectors (data not shown). Strikingly, HIF-1α induced by exposing mice to a hypoxic exposure (8% oxygen for 3 hr) declined rapidly to baseline levels by 2 hr after hypoxia (Figure 1E), in contrast to the sustained elevation seen after xenon preconditioning. These results indicate that xenon is an anesthetic gas with HIF-1α inducing capability and suggest that the mechanism is different from that of hypoxia, a common physiologic HIF-1α inducer.
Next we preconditioned HK2, a well-characterized human kidney proximal tubular epithelial cell line to xenon. HK2 cells exhibited HIF-1α activation with similar kinetics to those observed in the kidney in vivo (Figure 1F). Thus xenon preconditioning activates HIF-1α in isolated renal epithelial cells.

**Xenon preconditioning induces HIF-1α expression via the mTOR pathway.**

HIF-1α is regulated by ubiquitination of the α subunit; in the presence of oxygen these are hydroxylated, which permits capture by the VHL protein and subsequent destruction via the 26S proteasome. To ask whether xenon preconditioning activates HIF-1α by impairing destruction via this pathway, we used RCC4 cells which lack VHL, and an otherwise isogenic subline stably transfected with pcDNA3-VHL. As expected, RCC4 without VHL (RCC4−) cells expressed a higher level of HIF-1α compared to RCC4+ cells which are stably transfected with VHL. Both cell-lines showed a similar time-dependent increase in HIF-1α following xenon preconditioning. Thus, xenon-induced increase in HIF-1α expression is not dependent on VHL (Figure 2A). We also found that xenon preconditioning does not alter the amount of the PHD2 enzyme (prolyl hydroxylase domain containing 2) which is usually the most important HIF prolyl hydroxylase (Figure 2B). Therefore xenon preconditioning does not activate HIF via the canonical HIF-1α destruction pathway. HIF-1α abundance can be regulated at the level of gene transcription or mRNA translation. HIF-1α mRNA in cells preconditioned with xenon did not differ from those maintained under control conditions (Figure 2C). To determine the role of xenon preconditioning in modulating the translational efficiency of HIF-1α mRNA, we studied the effect of xenon preconditioning on the mammalian target of rapamycin (mTOR), a key integrator and
transducer of numerous intracellular signalling pathways and a well-described regulator of protein translation.\textsuperscript{11} Interestingly, preconditioning with xenon was found to increase expression of mTOR protein from 2 hr after the preconditioning stimulus, with maximal expression occurring at 24 hr after xenon preconditioning (5.93 ± 0.68 vs. 1.0 ± 0.1 of control, p < 0.001) (Figure 2D). To explore the significance of the increase in mTOR protein expression, we exposed cells to xenon in the presence of rapamycin (an inhibitor of mTOR), at 25, 50 and 100nM, and studied the protein expression of HIF-1\(\alpha\) 24 hr after xenon preconditioning. Rapamycin alone did not alter HIF-1\(\alpha\) expression when compared to control; as expected xenon preconditioning alone increased expression of HIF-1\(\alpha\) expression. However, the concurrent administration of rapamycin during xenon preconditioning significantly inhibited xenon-induced HIF-1\(\alpha\), with greatest HIF-1\(\alpha\) inhibition occurring at the highest concentration of rapamycin that we tested (100nM; 0.83 ± 0.21 vs. 2.83 ± 0.71, compared to xenon preconditioning alone) (Figure 2E). Xenon preconditioning also increased mTOR expression in mice kidneys as shown \textit{in vitro} (Figure 2F). We next administered rapamycin 1.5 mg/kg, i.p., to mice, just prior to the administration of xenon. As we had observed in the \textit{in vitro} study, rapamycin attenuated xenon-induced increase in HIF-1\(\alpha\) expression in the kidneys of mice (Figure 2G).

\textbf{HIF-1\(\alpha\) and EPO expression induced by xenon preconditioning is blocked by HIF-1\(\alpha\) siRNA administered intravenously.}

To determine whether preconditioning with xenon provides protection against renal ischemia followed by reperfusion in mice and whether this was mediated by HIF-1\(\alpha\), we developed a genetic knockdown approach since HIF-1\(\alpha\) knock out mice are not available owing to embryonic lethality.\textsuperscript{12} siRNA technology against HIF-1\(\alpha\) was
delivered by hydrodynamic injection. First, we verified delivery of synthetic double-stranded 3’-Fluorescein-labeled scrambled siRNA (50 µg in 1ml of PBS) into mouse tubular epithelium. Kidney tubular cells took up siRNA duplexes 24 hr after injection as shown by intracellular fluorescent signals while no such marker was found in PBS-injected control animals (Figure 3A, B); this result corroborates previous data that siRNA preferentially accumulates in the kidney. The functional effect of this efficient delivery was confirmed by significant knock down of HIF-1α and EPO mRNA (Figure 3C) and protein with no change in HIF-1β, which is constitutively expressed (Figure 3D,E).

**Xenon preconditioning protects against renal ischemic-reperfusion injury morphologically and functionally.**

Next, we determined whether preconditioning with xenon provides protection against renal ischemic-reperfusion injury (IRI) in mice when the renal pedicle of both kidneys was clamped for 25 minutes. Having shown the efficacy of siRNA at abrogating xenon-induced HIF-1α transcription, expression and downstream activity (Fig 3A-E), we investigated whether the renoprotective effect of xenon preconditioning could be abolished by knocking down HIF-1α. Twenty-four hours after IRI, kidneys and blood were harvested for histological assessment and for renal function (serum creatinine and urea), respectively. Morphological evidence of renal IRI injury was assessed using a histopathological scoring of cortical tubular damage by an investigator who was unaware of the experimental protocol. The histological score in the absence of xenon preconditioning was 259 ±17 which decreased to 54 ±19 (p < 0.01) and 69 ±20 (p < 0.01) in PBS or negative control siRNA injection with xenon preconditioning respectively; pretreatment with HIF-1α siRNA injection abrogated the renoprotective
effect of xenon preconditioning (284 ± 6; p > 0.05) (Figure 4A). Qualitatively similar changes were noted in renal function; following IRI; notably, the plasma creatinine (Cr) rose from 35 ± 1.2µM/L to 162 ± 18 µM/L. Prior exposure to xenon preconditioning was protective, with creatinine showing no significant difference from animals not subjected to renal injury. When xenon preconditioning was preceded by HIF-1α siRNA injection this abrogated the protective effect so that creatinine was significantly (p < 0.05) higher (91 ± 58) than that seen in the uninjured animals (Figure 4B). Changes in plasma urea were similar (Figure 4C).

In order to evaluate kidney damage at the cellular level, TUNEL staining was used to detect apoptotic tubular cells. Quantitatively, TUNEL positive cells were significantly decreased to 82 ± 27 (p < 0.01) and 155 ± 16 (p < 0.01) in mice receiving PBS or negative siRNA injection followed by xenon preconditioning respectively from 297 ± 32 in the non-preconditioned IRI mice. The latter was not significant when compared to the value of 272 ± 28 (p > 0.05) in mice treated with HIF-1α siRNA injection followed by xenon preconditioning (Figure 4D).

Xenon preconditioning protects from renal failure.

To assess whether xenon preconditioning was also effective in the context of a more severe insult to renal function (Table 1), we performed further experiments in which the right renal pedicle was clamped for 40 min and the left kidney was removed. Long-term survival (no signs of illness at 7 days) was noted in 75% of animals preconditioned with xenon prior to IRI. In contrast, animals not preconditioned with xenon, or preconditioned with xenon combined with HIF-1α siRNA fared much worse. Within 2 days, 38% of these animals were dead (either spontaneously or
euthanised because of severe ill health measured against defined criteria) and no animals survived beyond 5 days post-IRI (p < 0.001 vs xenon preconditioning group) (Figure 5A). The mean value of plasma creatinine and urea rose more than 10-fold at day 2 (just prior to termination) following IRI. Animals treated with xenon preconditioning exhibited normal creatinine and urea values even at 7 days after IRI (Figure 5B, C).

Discussion
This study demonstrates for the first time that, under normoxic conditions, xenon preconditioning induces a sustained increase of HIF-1α activity in adult mouse kidney and in a human kidney cell line through increased translational efficiency of HIF-1α involving the mTOR pathway. The xenon-induced HIF-1α expression prevents apoptosis, renal tubular damage, renal dysfunction and renal failure following varying degrees of ischemic reperfusion injury and its effect is abolished with HIF-1α siRNA.

HIF-1 regulates the adaptive response to hypoxia and other stresses by orchestrating the transcription of genes involved in a wide variety of cell processes - from glycolysis to angiogenesis - that promote the switch to a more tolerant phenotype. HIF-target genes have themselves already been implicated in mediating protection against IRI.15 EPO upregulation during ischemic acute renal failure reduces anemia and increases renal tubular regeneration.16 Therefore, the manipulation of the HIF-1α pathway appears to be a promising strategy for minimising renal IRI by disabling HIF-1α degradation with a PHD inhibitor5 or cobalt chloride.17 The data reported here clearly demonstrate that xenon preconditioning is a natural physiological HIF-1α inducer and protects kidneys against IRI by the up-regulation of HIF-1α and EPO.
We now demonstrate an alternative strategy to increase HIF activity through mTOR, a large evolutionarily conserved protein kinase that plays a pivotal role in regulating mRNA translation and thereby effecting cell growth and survival. mTOR phosphorylates the translational inhibitor 4E-BP that leads to its inactivation and subsequent loss of its inhibitory effects on the cap-binding protein eIF-4E, a necessary component of the translation initiation complex that can activate S6 kinase. S6 kinase activity leads to the recruitment of ribosomal proteins S6 and eIF-4B and these stimulate the translation of mRNA containing terminal oligopyrimidine tract 5′TOP) motifs in the 5′ untranslated region, including HIF-1α mRNA. Upregulation of HIF-1α in response to growth factor signaling via the PI3K/Akt/mTOR pathway provides a route by which proliferating cells make adaptive metabolic changes and produce angiogenic growth factors, while inhibition of mTOR provides a route to decrease HIF-1α activation in neoplasia. While we have not formally investigated how xenon preconditioning increases mTOR activity in the kidney, the effects of xenon preconditioning on PI3K and AKT, two upstream regulators of mTOR, have been noted in the brain (unpublished observation).

Whether HIF-1 has direct protective effects in renal tubular cells has been questioned; cisplatin-induced cell apoptosis was demonstrated to be ameliorated by hypoxic exposure, but not by cobalt chloride exposure which increases HIF expression. However, Tanaka et al did demonstrate amelioration of cisplatin-induced cell apoptosis by cobalt chloride administration in both in vitro and in vivo models. Therefore, the role of HIF in the protective effect of hypoxia is not fully resolved.
Our current study may have significant clinical implications as AKI is still an important contributor to patient morbidity and mortality following major vascular surgical interventions and renal transplantation.\(^1\) Xenon preconditioning displays many of the favorable properties of the ideal inhalational anesthetic. It is a remarkably safe anesthetic with a fast induction and emergence, due to its very low blood gas partition coefficient.\(^8\) Furthermore, it is the ideal “green” gas since it is returned to the atmosphere underivatized. As the mortality rate of patients with postoperative acute kidney failure requiring hemodialysis is as high as 60%,\(^1\) there is a critical need to develop therapeutic strategies to enhance tolerance of the kidney in withstanding ischemic injury. As demonstrated here, xenon-induced HIF-1\(\alpha\) expression prevents renal IRI injury and this finding should be considered as a therapeutic strategy to be extended for clinical use. However, the renoprotective effect of xenon-induced changes of HIF-1\(\alpha\) expression at the earlier time, e.g. 2 hr post-exposure (as shown in Figure 1A), remains unknown. Nevertheless, if our data can be extrapolated to clinical settings, xenon preconditioning may serve an important protective function when administered prior to a variety of elective procedures in which the cessation of renal blood supply is inevitable.

**CONCISE METHODS**

**Animals**

Ten-week-old male C57BL/6J mice weighing 20-25g were housed in temperature and humidity-controlled cages with free access to sterile acidified water and irradiated food in a specific pathogen-free facility at Imperial College London. All procedures were performed strictly under the United Kingdom Animals (Scientific Procedures) Act 1986.
siRNA injection
Deprotected and annealed HIF-1α or scrambled siRNAs with or without a 3’-Fluorescein-label (Qiagen, Crawley, West Sussex, UK) were dissolved in siRNA suspension buffer and further diluted in RNase-free PBS before use. HIF-1α siRNA was produced by annealing r(GCU CAA UUU AUG AAU AUU A)dTdT (sense) and r(UAA UAU UCA UAA AUU GAG C)dGdG (antisense). For hydrodynamic injection, HIF-1α or scrambled siRNA (negative control) (50 µg in 1 ml of PBS) or 1 ml of PBS was rapidly injected (within 15 sec) into a tail vein via an acutely-sited cannula followed by 0.3 ml of PBS flush under volatile anesthesia.

Gas exposure
Mice were exposed to either 70% xenon, 70% nitrogen (N₂) or 70% nitrous oxide (N₂O) balanced with 30% oxygen (O₂) for 2 hr or 8% oxygen balanced with N₂ for 3 hr via an anesthetic chamber. Gas concentrations of xenon, O₂ and N₂O were monitored by xenon monitor (Air Products Model No. 439Xe, Bradford, UK) and Datex monitor (Datex-Ohmeda, Bradford, UK).

Kidney ischemia-reperfusion
Twenty-four hr after gas exposure, kidney ischemia-reperfusion injury was induced either by bilateral renal pedicle clamping for 25 min or by right renal pedicle clamping for 40 min and left nephrectomy. Sham-operated mice had dissection as above but with no occlusion of the renal vessel. The intra-abdominal temperature was maintained at 36 ± 0.1 °C with a heating pad which was servo-adjusted by a temperature controller (Engineering Inc, Stamford, CT, USA) throughout the
experiment. For survival experiments, mice were monitored on a daily basis with a scoring assay based on body weight, activity and general appearance (Table 1). Any animals that scored > 7 were euthanized.

**Cell line**

HK-2 cells are derived from adult human kidney proximal tubular epithelial cells. RCC4- and RCC4+ refer to two derivatives of the VHL defective renal carcinoma cell line RCC4. RCC4+ is stably transfected with an expression plasmid encoding wild type VHL and RCC4- with an empty vector. They were cultured at 37°C in RPMI 1640 medium supplemented with 10% foetal bovine serum, 2mM L-glutamine, and 100U/mL penicillin streptomycin (Invitrogen) in a humidified air/ 5% CO₂ atmosphere. They were used soon after reaching 80% confluence.

**Real time RT-PCR**

Total RNA was isolated from harvested kidneys 0, 2, 4, 8 and 24 hours after xenon preconditioning using the RNeasy Mini Kit (Qiagen) according to the manufacturer’s protocol, followed by quantification based on ultraviolet absorption at 260nm. Kidneys from mice exposed to 70% nitrogen balanced with oxygen were used as controls. Extracted RNA was then converted to cDNA using SuperScript II reverse transcriptase (Invitrogen) for single-stranded cDNA synthesis. The cDNA product was used for real-time PCR in the presence of SYBR Green PCR Master Mix (Roche Diagnostics Ltd). The amplification was performed in a RotorGene Sequence Detector (Corbett Research) at 95°C for 10 minutes, followed by 45 cycles of 95°C for 20 s, 58°C for 20 s and 72°C for 20 s. Gene-expression levels were assessed by the comparative threshold method, using β-actin as the internal control. PCR primers
(Invitrogen) were designed with sequences as follows: HIF-1α forward, 5’-TCAAGTCAGCAACGTGGAAG-3’; reverse, 5’-TATCGAGGCTGTGCTCGACTG-3’; EPO forward 5’-AGTCGCGTTCTGGAGAGGTA-3’; reverse 5’-AGGATGGCTTCTGAGAGCAG-3’; β-actin forward 5’-AGCCATGTACGTAGCCATCC-3’, reverse 5’-CTCTCAGCTGTTGGTTGAA-3’.

PCR products were separated by electrophoresis on a 2% agarose gel containing the fluorescent dye ethidium bromide, and visualized via a Fluor-S Multi-Imager (BioRad) for illustration purposes or the ratio of HIF-1α mRNA/β-actin mRNA were calculated and then normalized with control for quantitative analysis.

**Immunoblot**

Proteins were extracted from the cell lines or renal cortices at 0, 2, 4, 8 and 24 hours after xenon preconditioning by cell disruption in cell lysis buffer (Cell Signalling) and sonication with an ultrasonic probe, followed by centrifugation at 10,000 g for 10 minutes at 4°C. The supernatant was collected for Western blotting. Samples containing 30µg of extracted protein, as determined by the Bradford protein assay (BioRad), were loaded on a NuPAGE 4-12% Bis-Tris gel (Invitrogen) for protein fractionation by electrophoresis and then electro-transferred to a nitrocellulose membrane (Hybond ECL; Amersham Biosciences). Blots were blocked with 5% non-fat dry milk in TBS (pH8.0, containing 0.1% Tween-20), and probed with appropriate antibodies followed by HRP-conjugated secondary antibodies and visualisation with enhanced chemiluminescence (Cell Signalling).

**Histologic score**
The sum score was calculated from the analysis of 10 cortical tubules/cross-section stained with H&E (10 sections/kidney) by using a modified scoring system;\textsuperscript{13} 0, no damage. 1, mild damage: rounded epithelial cells and dilated tubular lumen. 2, moderate damage: flattened epithelial cells, loss of nuclear staining and substantially dilated lumen; 3, severe damage: destroyed tubules with no nuclear staining of epithelial cells.

**Plasma creatinine and urea**

Both creatinine and urea were measured in 100 µl of plasma with an Olympus AU640 analyzer (Diamond Diagnostics, Watford, UK).

**TUNEL staining**

Apoptosis of tubular epithelial cells was detected by in situ TUNEL assay (Obiogene) according to the manufacturer’s instructions. The fixed cryostat sections were washed with PBS and then treated with proteinase K 20 µg/ml at room temperature for 15 min. For positive controls sections were treated with nuclease (R&D system) at 37°C for 15 min. The sections were quenched in 3% hydrogen peroxide in PBS for 5 min. The quenched sections were labelled with TDT enzyme at 37°C for 1 hour in a humidified chamber and subsequently incubated with anti-digoxigenin conjugated to horseradish peroxidase for 30 mins at room temperature. They were then stained with DAB followed by counterstaining with methyl green. The sum of TUNEL\textsuperscript{+} cells in an objective grid from 10 areas of randomly selected renal cortex was counted under a 40× objective lens by an investigator who was blinded to the experimental protocol.
**Statistical analysis** Statistical comparison was by ANOVA followed by post hoc Student-Newman Keul’s test where appropriate. Survival was analyzed by Kaplan-Meier test. A $p < 0.05$ was considered as statistically significant.

**ACKNOWLEDGMENTS**

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**DISCLOSURES**

M.M. is paid consultant for Air Products, a company that is interested in developing clinical applications for medical gases, including xenon.
REFERENCES


**Figure legends**

**Figure 1.** Xenon activates HIF in the mouse kidney in vivo and in cultured HK2 cells. Mice were exposed to 70% nitrogen (N₂), 70% xenon (Xe), 70% nitrous oxide (N₂O), for 2 hr. As a positive control for HIF activation mice were exposed to 8% oxygen. Renal cortices were harvested for immunoblot from 0 hr to 24 hr after treatments. (A) Xenon significantly increased HIF-1α expression from 2 hr after exposure (n = 5-6 at each time point) compared to N₂ control. (B) Xenon significantly increased EPO expression (n = 5 - 6) 24 hr after exposure compared to N₂ (control). (C) Xenon significantly increased expression VEGF expression (n = 5 - 6) from 8 hr after exposure compared to N₂ control. (D) Exposure to the anesthetic gas, N₂O, did not affect HIF-1α expression (n = 4). (E) The effect of hypoxia on HIF-1α expression (n = 5) was not significant 2 hr or more after exposure compared to N₂ control. (F) Xenon significantly increases HIF-1α expression from 8 hr after exposure (n = 4 at each time point) compared to N₂ control in HK2 cell line. Data are expressed as mean ±SD. C, control. *p < 0.05; **p < 0.01 vs control.

**Figure 2.** Exploration of mechanisms for increased level of HIF-1α protein. For in vitro experiments, RCC4+VHL were exposed to 70% xenon balanced with oxygen for 2 hours and harvested at 0 – 24 hours following this. (A) Exposure to xenon increases HIF-1α expression whether the VHL ubiquitylation process is present.
(RCC4+ cells) or absent (RCC4- cells) (an example of three independent experiments).

(B) *In vivo* effects of HIF prolyl hydroxylase domain-containing 2 (PHD2). Exposure to xenon does not increase expression of the enzyme, HIF PHD2 in RCC4+VHL cells (n = 4). (C) RT-PCR revealed that exposure to xenon did not alter the level of HIF-1α mRNA (n = 4). (D) Xenon exposure significantly increased expression of the protein mammalian target of rapamycin (mTOR) after 24 hours (n = 4). C = control. *p < 0.05; **p < 0.01 ***p < 0.001 vs control. (E) Rapamycin, an mTOR inhibitor, attenuates xenon-induced HIF-1α expression 24 hours after exposure. C, control; R, rapamycin; Xe, xenon. *p < 0.05; **p < 0.01 vs xenon. (n = 4). (F) Xenon exposure caused a time-dependent increase expression of the protein mammalian target of rapamycin (mTOR) in the mouse kidney (an example of three independent experiments). (G) In vivo, pretreatment with rapamycin 1.5 mg/kg i.p. prevented enhancement of HIF-1α expression (an example of three independent experiments). Data are expressed as mean ± SD. C, control.

**Figure 3.** In vivo effects of HIF-1α siRNA. (A) Injection of PBS and (B) fluorescein-labeled scrambled siRNA reveals that siRNA is present in the renal parenchyma 24 hr after injection. (C) 24 hr following treatment with HIF-1α siRNA, expression (assessed by real time RT-PCR) of HIF-1α and its downstream effector (erythropoietin [EPO]) was decreased. Expression of (D) HIF-1α (but not HIF-1β) and (E) erythropoietin (EPO) induced by xenon exposure was decreased 24h following treatment with HIF-1α siRNA (n = 5). Data are mean ± SD. C, control. *p < 0.05.
**Figure 4.** In vivo renoprotection by xenon vs renal ischemia-reperfusion injury (IRI). Mice were pretreated with hydrodynamically injected siRNA (or scrambled siRNA or PBS) and 24 hr later were exposed to 70% nitrogen (N\(_2\)) or 70% xenon (Xe) balanced with oxygen (O\(_2\)) for 2 hr. IRI was by bilateral clamping of the renal pedicle for 25 min, 24 hr after gas exposure. (A) Quantification of histological scoring following IRI in mice pretreated and exposed to various gas mixtures treatments (n = 6). Attenuation by HIF 1α siRNA pretreatment of the renoprotective effect of xenon in IRI as reflected by (B) plasma creatinine and (C) plasma urea (n = 6). (D) Mean value of TUNEL\(^+\) cells in renal sections following various treatments (n = 6). C, control; Xe, xenon; NegsiR, negative siRNA; HIFsiR, HIF-1α siRNA. Data are mean ±SD. *p < 0.05; **p < 0.01 vs control.

**Figure 5.** Xenon protects from renal failure and this is HIF dependent. Mice were given a hydrodynamic injection of PBS or specified siRNA and 24 hr later they were exposed to 70% nitrogen (N\(_2\)) or 70% xenon (Xe) balanced with oxygen (O\(_2\)) for 2 hr. Kidney injury was induced by clamping the right renal pedicle for 40 min. The contralateral kidney was removed 24 hr after exposure. (A) Kaplan-Meier survival curve. (B) Plasma creatinine and (C) urea from the mice with PBS or siRNA injection prior to xenon preconditioning (or control) followed by ischemia-reperfusion challenge. Samples were taken at the times indicated when animals were euthanised. Blood samples were not available from 3 and 2 animals terminated during post-experimental day 3-7 in the sham preconditioning and HIF-1α siRNA + xenon preconditioning group respectively. C, control; Xe, xenon; NegsiR, negative control siRNA; HIFsiR, HIF-1α siRNA. Data are mean ±SD. C, control. **p < 0.01.
Table 1: Post-operative assessment

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<td>1</td>
</tr>
<tr>
<td>&gt; 80-90% pre-op weight</td>
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Animals will be killed when the score is > 7.
Fig. 5