Defective postreperfusion metabolic recovery directly associates with incident delayed graft function

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Delayed graft function (DGF) following kidney transplantation affects long-term graft function and survival and is considered a manifestation of ischemia reperfusion injury. Preclinical studies characterize metabolic defects resulting from mitochondrial damage as primary driver of ischemia reperfusion injury. In a comprehensive approach that included sequential establishment of postreperfusion arteriovenous concentration differences over the human graft, metabolomic and genomic analysis in tissue biopsies taken before and after reperfusion, we tested whether the preclinical observations translate to the context of clinical DGF. This report is based on sequential studies of 66 eligible patients of which 22 experienced DGF. Grafts with no DGF immediately recovered aerobic respiration as indicated by prompt cessation of lactate release following reperfusion. In contrast, grafts with DGF failed to recover aerobic respiration and showed persistent adenosine triphosphate catabolism indicated by a significant persistently low post reperfusion tissue glucose-lactate ratio and continued significant post-reperfusion lactate and hypoxanthine release (net arteriovenous difference for lactate and hypoxanthine at 30 minutes). The metabolic data for the group with DGF point to a persistent post reperfusion mitochondrial defect, confirmed by functional (respirometry) and morphological analyses. The archetypical mitochondrial stabilizing peptide SS-31 significantly preserved mitochondrial function in human kidney biopsies following simulated ischemia reperfusion. Thus, development of DGF is preceded by a profound post-reperfusion metabolic deficit resulting from severe mitochondrial damage. Strategies aimed at preventing DGF should be focused on safeguarding a minimally required post-reperfusion metabolic competence.

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Delayed graft function (DGF), the phenomenon of deferred functional recovery of a donor graft following transplantation, has detrimental effects on long-term graft function and graft survival.[1–3] The incidence of DGF is steadily rising, a fact thought to reflect increased use of so-called marginal organs in an era of donor shortages. DGF incidences up to 70% are reported for deceased donor grafts.[2,4]

Incident DGF is thought to largely reflect ischemia/reperfusion (I/R) injury,[5,6] the increase of tissue damage following reperfusion of previously ischemic tissue. A range of pharmaceutical interventions that target I/R such as anti-oxidants and anti-inflammatory and immune-modulatory drugs successfully quench I/R injury in preclinical models, but efforts to translate these experimental findings to the human situation have been unsuccessful.[5,7] Therefore, there is currently no intervention that alleviates DGF and other forms of clinical I/R injury, a notion that points to an impaired translatability of preclinical findings.[5]

Although DGF is common in deceased donor grafts, it is rare in the context of living donor kidney transplantation. This is a notable observation, because these grafts are also exposed to several hours of ischemia prior to reperfusion.[8] It was thus reasoned that differences in the response to I/R between living and deceased donor grafts provide critical clues toward the mechanism(s) driving DGF. Living donor procedures were used as comparators. We and others previously excluded commonly implicated causative factors such as oxidative damage;[9] neutrophil,[10] thrombocyte,[11,12] or complement activation;[12–15] and inflammation[12,16,17] as main drivers of clinical I/R injury in the context of kidney

transplantation. These observations imply that clinical I/R injury is driven by factors beyond those commonly brought forward.

There is accumulating evidence for a role of metabolic dysfunction as driver of I/R injury particularly from the context of myocardial I/R.[18–21] However, it is unclear whether and how these observations translate to the context of kidney transplantation, and in particular to incident DGF.

We here show that incident DGF is associated with profound and persistent postreperfusion metabolic deficit caused by severe mitochondrial damage. The consequent severe metabolic shortfall interferes with processes critical for cell homeostasis and recovery such as gene transcription. Importantly, it was observed that mitochondrial damage could be partially rescued by the archetypical mitochondria stabilizing peptide SS-31.[22] The potential of this cardiolipin-binding peptide is extensively shown in preclinical studies[22,23] and the compound has now entered clinical evaluation.[24]

RESULTS

Characteristics of the patient groups are summarized in Table 1 and Supplementary Figure S1. This report is based on sequential studies in which a total of 85 patients were enrolled. Twelve patients refused to give informed consent and 7 patients were excluded due to cancelled surgery. Postoperative course was uneventful in all patients. One-year patient and graft survival was 100%. All living donor procedures showed immediate renal functional recovery. More than 50% of the deceased donor grafts developed DGF.

Tissue and plasma metabolites

We first assessed net lactate release from the reperfused graft as a readout of metabolic competence. Figure 1 shows arterial (red) and venous (blue) lactate levels over the reperfused kidney. Reperfused living donor grafts and deceased donor grafts without delayed graft function (–DGF grafts) show an almost instantaneous converging of arterial and venous plasma lactate levels, indicating immediate cessation of lactate release following washout of accumulated lactate. In contrast, persistent net lactate release (net graft lactate release 30 minutes after reperfusion [mean ±SEM]: 1.7 ± 0.67 mmol in grafts with DGF [+DGF] vs. 0.0 ± 0.04 and 0.0 ± 0.05 in respective living and –DGF grafts [$P < 0.000038$]) and persistent metabolic acidosis (venous pH: 7.22 ± 0.06 in +DGF grafts vs. 7.33 ± 0.02 and 7.33 ± 0.0215 in respective living and –DGF grafts [$P < 0.004$]) were observed for grafts with delayed graft function (+DGF grafts) (Supplementary Figure S2).

Data from the renal tissue biopsies followed these observations with recovery of tissue glucose content and negligible lactate in biopsies taken 45 minutes after reperfusion in living donor grafts (glucose-lactate ratio: before 0.19 ± 0.03 vs. after 0.90 ± 0.16 reperfusion [$P < 0.0039$] and –DGF grafts: 0.28 ± 0.07 vs. after 0.87 ± 0.24 reperfusion [$P < 0.026$]) (Figure 2a). In contrast, +DGF grafts showed persistent high tissue lactate and absent glucose recovery (before 0.21 ± 0.04 vs. after 0.22 ± 0.06 reperfusion) (Figure 2a), resulting in a persistent low glucose-lactate ratio. These observations for lactate and glucose may indicate a temporal dominance of glycolysis as the dominant source of adenosine triphosphate (ATP) in +DGF grafts or alternatively (and nonexclusively) a situation of metabolic exhaustion. To test the latter, we assessed arteriovenous (AV)-differences for hypoxanthine, the end product of ATP catabolism. Figure 2b shows the hypoxanthine washout and immediate cessation of hypoxanthine release in living donor grafts and –DGF grafts, but persistent hypoxanthine release ($P < 0.0024$) from +DGF grafts (12.1 ± 4.63 µmol hypoxanthine in +DGF grafts vs. 0.0 µmol ± 0.67 and 0.6 µmol ± 0.53 in living and –DGF grafts). This observation points to ongoing postreperfusion ATP catabolism in +DGF grafts.

Gene expression profiles

From these findings, a picture emerges of failing metabolic recovery as a critical determinant of later DGF. Given the high metabolic demands of gene transcription,[25] we used the postreperfusion transcriptome as a readout of metabolic competence. We first established early reperfusion-related changes in transcriptome of living donor grafts (i.e., gene expression profiles in paired kidney biopsies taken immediately before and 45 minutes after reperfusion) as a reference. Functional analysis (ingenuity pathway analysis platform) showed that early reperfusion of living donor grafts is dominated by up-regulation of redox response networks and a broader, more moderate up-regulation of predominantly

Table 1 | Patient characteristics

	Donor			Recipient		
Group	Donor type (%)	Age (yr)	Male (%)	Age (yr)	Male (%)	Cold ischemia time (min)
Living ($n = 27$)	Living 100	53.3 ± 1.7	44	50.7 ± 2.8	63	217.3 ± 3.7
–DGF ($n = 17$)	DBD = 58 DCD = 42	53.3 ± 4.6	53	58.1 ± 2.4	58	791.9 ± 45.3
+DGF ($n = 22$)	DBD = 27 DCD = 73	53.4 ± 3.2	73	56.6 ± 2.8	59	996.9 ± 60.0

Ages and cold ischemia time are expressed as mean ± SEM. Patients were included in 3 recruiting rounds (see flow chart in Supplementary Figure S1). Recipients of living donor grafts were taken as a reference because DGF is rare in this group. Deceased donor grafts were classified based on outcome after transplantation. DGF is the status in which the transplant recipient is in need of dialysis in the first week(s) after transplantation and is primarily caused by ischemia/reperfusion injury. Duration of cold ischemia was significantly different between groups (analysis of variance: $P < 0.01$).
DBD, donation after brain death; DCD, donation after cardiac death; +DGF, with delayed graft function; –DGF, without delayed graft function.