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Cytokine phenotype, genotype, and renal outcomes at cardiac surgery ☆,☆☆

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ABSTRACT

Background: Cardiac surgery modulates pro- and anti-inflammatory cytokine balance involving plasma tumour necrosis factor alpha (TNFα) and interleukin-10 (IL-10) together with urinary transforming growth factor beta-1 (TGFβ1), interleukin-1 receptor antagonist (IL1ra) and tumour necrosis factor soluble receptor-2 (TNFsr2). Effects on post-operative renal function are unclear. We investigated if following cardiac surgery there is a relationship between cytokine (a) phenotype and renal outcome; (b) genotype and phenotype and (c) genotype and renal outcome. Since angiotensin-2 (AG2), modulates TGFβ1 production, we determined whether angiotensin converting enzyme insertion/deletion (ACE I/D) genotype affects urinary TGFβ1 phenotype as well as renal outcome.

Methods: In 408 elective cardiac surgery patients we measured pre- and 24 h post-operative urinary TGFβ-1, IL1ra and TNFsr2 and pre- and 2 h post-operative plasma TNFα and IL-10. Post-operative responses were compared for each cytokine in patients grouped according to presence or absence of renal dysfunction defined as a drop from baseline eGFR of greater than 25% (as calculated by the method of modification of diet in renal disease (MDRD)) occurring (1) within the first 24 and (2) 48 postoperative hours (early renal dysfunction), (3) on the fifth postoperative day (late renal dysfunction) or (4) at any time throughout the 5 day postoperative period (early and late combined). Patient genotype was determined for TNF/G-308A, TGFβ1-509 C/T, IL10/G-1082A and ACE I/D.

Results: Post-operative plasma IL-10 and urinary TGFβ1 responses were significantly higher in patients who developed early renal dysfunction. IL1ra and TNFsr2 responses were significantly lower 24 h post-operatively in patients who developed late renal dysfunction. Genotype did not alter cytokine phenotype or outcome.

Conclusions/inferences: Cytokine profiling may help predict early and late renal dysfunction. Genotypes studied did not alter phenotype or outcome.

1. Introduction

Cardiac surgery increases the plasma proinflammatory mediators tumour necrosis factor-α (TNFα) and interleukin-8 (IL-8) [1]. There arises a compensatory plasma anti-inflammatory cytokine response in the first 3 postoperative hours, namely IL-10, followed by interleukin-1 receptor antagonist (IL1ra) and over the next two days tumour necrosis factor soluble receptor 1 and 2 (TNFsr1 and 2) [1]. There is a parallel urinary anti-inflammatory response with increased urinary IL1ra, TNFsr2 [2,3] and transforming growth factor beta-1 (TGFβ1) [3]. However, TNFα and IL-1β though filtered by the kidneys are removed from urine through absorption in the proximal tubules and denaturing by intracellular proteolytic mechanisms [4].

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1.1. Phenotype and outcome

It has been suggested that the urinary anti-inflammatory response allows safe plasma pro-inflammatory renal filtration such as TNFα [5]. However, these studies were inadequately powered to demonstrate any relationship between these cytokine changes and later renal dysfunction as estimated by glomerular filtration rate (eGFR) reduction. Other biomarkers recently profiled to predict early onset renal dysfunction include proinflammatory neutrophil gelatinase associated leukocalin (NGAL) and interleukin-18 (IL-18) [6,7].

We hypothesize that perioperative cytokine profiling involving anti-inflammatory cytokines can help predict postoperative renal dysfunction. In particular patients who develop clinically significant renal dysfunction after cardiac surgery will have significantly lower urinary anti-inflammatory cytokine production than those who do not. Since *in vitro* and *in vivo* studies of acute kidney injury (AKI) pathogenesis have suggested a reno-toxic role for TNFα [8,9] and renal protective roles for TGFβ1 [10] and IL-10 [11] we wished to determine if changes in these cytokines are related to clinically significant postoperative renal dysfunction.

1.2. Genotype relationship to phenotype and outcome

Patient genotypes influencing clinical outcomes have been described at cardiac surgery [12,13]. Mechanisms by which genotype alters clinical outcome are unclear. Galley et al described the IL-10 hypo-secretor genotype IL-10-1082AA [13] (which is linked with poorer 24 h outcomes) being associated with higher peri-operative IL-10 responses than the IL-10 hyper-secretor genotype, challenging the traditional concept of gene related outcomes arising mainly from gene related differences in magnitude of protein production. Since the cytokines TNFα [8,9,14], TGFβ1 [10] and IL-10 [11] have been linked with the pathogenesis of renal dysfunction we wished to investigate the hypothesis that perioperatively there is for these 3 cytokines a relationship between (a) genotype and phenotype; and (b) genotype and renal outcome. In addition, since angiotensin converting enzyme insertion/deletion (ACE I/D) polymorphism has already been linked with renal outcomes in African–Americans [12] and angiotensin-2 (AG2) alters TGFβ1 production [15], we hypothesized that in our population there is a relationship between ACE I/D genotype and urinary TGFβ1 phenotype as well as between ACE I/D genotype and renal outcome.

1.3. Detailed justification of the polymorphisms and cytokines studied

1.3.1. TNF/G-308A

G–A polymorphism at position −308 on the TNFα gene promoter, is associated with increased transcription [16,17] and production [17,18] of the TNFα gene *in vitro*. Since TNFα/G-308A(A/A) has been linked with greater renal complications in other patient populations [8,9,14] we wished to determine if the hypersecretor genotype (A/A) conferred increased risk of post-operative renal dysfunction as well as influencing the magnitude of the plasma TNFα response at cardiac surgery.

1.3.2. IL10/G-1082A [19]

G to A polymorphism (alleles IL-10 1∗G and IL-10 1∗A, respectively) at position −1082 in the IL-10 gene promoter correlates with lower IL-10 production in stimulated lymphocytes *in vitro* [17,19,20,21] while the GG polymorphism had higher constitutive mRNA and serum IL10 in healthy subjects [22]. Moreover, the IL-10 intermediate/high producer genotype (−1082 G-allele carrier) (G/G) was associated with lower risk of death in patients with AKI [9]. Furthermore, IL-10 is linked with preservation of renal function [11]. These findings are consistent with IL10-1082GG (hypersecretor) being associated with better 24 h outcomes after cardiac surgery [13]. Although these papers suggest that IL-10 may be protective against peri-operative renal dysfunction paradoxically Galley et al. found that the greater the IL-10 response after cardiac surgery, the greater the risk of organ dysfunction at 24 h and of note they found that it was the IL-10-1082GG (hyper-secretor) polymorphism which was associated with lower IL10 responses after CPB [13].

To clarify these apparent contradictory reports this study investigates the hypothesis that the IL10/G-1082A (G/G) (hyper-secretor) polymorphism is associated with significantly lower incidence of renal complications as well as influencing the magnitude of the plasma IL-10 response.

1.3.3. ACE I/D

The ACE I/D DD genotype is associated with higher levels of circulating ACE than the ID or II genotypes and is more frequent in patients with myocardial infarction than controls [23]. Patients with chronic renal dysfunction who are AG2 hypersecretors (ACE D/D) have heightened progression to dialysis dependent renal failure [24] unless treated with ACE inhibitors [25]. The rationale is that AG2 through enhanced TGFβ1 delivers healing signals to the kidney which in chronic renal dysfunction leads to progressive scar tissue formation and dialysis dependence [15]. While TGFβ1 in chronic renal dysfunction is harmful to renal function through progressive unwanted scar formation, in the context of acute renal injury the converse may be true as illustrated by a model of hydrogen peroxide induced HK2 cell necrosis where exogenously administered TGFβ1 induced tubular protection [10]. Accordingly, we investigated the hypothesis that in the context of acute peri-operative renal dysfunction, possession of the AG2 hypersecretor (ACE D/D) genotype lessens risk of acute peri-operative renal dysfunction. Corroborative evidence would include AG2 receptor blockade heightening risk of acute peri-operative renal dysfunction.

1.3.4. TGFβ1-509 C/T

TGFβ1 negatively regulates cell proliferation. TGFβ1-509 TT genotype is hyper-secretor [26] with plasma levels of TGFβ1 being twice as high in TGFβ1 −509 T homozygotes as in −509 C homozygotes [27]. We investigated whether TGFβ1 509 T/T (hyper-secretor) genotypes have higher urinary TGFβ1 production than C/C homozygotes and if TT hyper-secretor genotypes have reduced renal dysfunction risk.

2. Methods

2.1. Patients and recruitment

Four hundred and eight consecutive patients undergoing elective cardiac surgery were studied. The patients were recruited within the Cardiac Surgical Unit of the Royal Victoria Hospital Belfast, Northern Ireland ($n = 304$) and Papworth Hospital NHS Foundation Trust in Cambridge, England ($n = 104$). Local ethical committee approvals were received and written informed patient consent was obtained. Exclusion criteria included preoperative dialysis dependent renal failure or significant renal disease (eGFR < 40 ml min^{-1}) and diabetes mellitus. Patients on pre-operative angiotensin conversion enzyme (ACE) inhibitor therapy were not excluded. The anaesthetic technique in both centers was based on the use of propofol and fentanyl. Isoflurane was used in most patients either as an adjunct anaesthetic agent or to control blood pressure. Pancuronium provided muscle relaxation. Post-operative analgesia was with morphine infusion.