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Bilirubin, a new therapeutic for kidney transplant?

Vikram L. Sundararaghavan^{a,b}, Sivjot Binopal^c, David E. Stec^d, Puneet Sindhwani^a, and Terry D. Hinds Jr.^{a,b,*}

^aDepartment of Urology and Renal Transplant, Toledo, OH 43614, USA

^bCenter for Hypertension and Personalized Medicine, Department of Physiology & Pharmacology, University of Toledo College of Medicine, Toledo, OH 43614, USA

^cInternal Medicine Department, Kettering Medical Center, Kettering, OH 45429, USA

^dDepartment of Physiology & Biophysics, Mississippi Center for Obesity Research, University of Mississippi Medical Center, Jackson, MS 39216, USA

Abstract

In patients with end-stage renal disease, kidney transplantation has been associated with numerous benefits, including increased daily activity, and better survival rates. However, over 20% of kidney transplants result in rejection within five years. Rejection is primarily due to a hypersensitive immune system and ischemia/reperfusion injury. Bilirubin has been shown to be a potent antioxidant that is capable of potentially reversing or preventing damage from reactive oxygen species generated from ischemia and reperfusion. Additionally, bilirubin has several immunomodulatory effects that can dampen the immune system to promote organ acceptance. Increased bilirubin has also been shown to have a positive impact on renal hemodynamics, which is critical post-transplantation. Lastly, bilirubin levels have been correlated with biomarkers of successful transplantation. In this review, we discuss a multitude of potentially beneficial effects that bilirubin has on kidney acceptance of transplantation based on numerous clinical trials and animal models. Exogenous bilirubin delivery or increasing endogenous levels pre- or post-transplantation may have therapeutic benefits.

Keywords

Bilirubin; Obesity; Oxidative stress; Renal transplant; Gilbert's syndrome

1. Introduction

Kidney disease is a common co-morbidity in patients who are obese or diabetic, which may ultimately progress to end-stage renal disease (ESRD). According to the U.S. Renal Data System Annual Report, >660,000 Americans are being treated for ESRD. Treatment for

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*Corresponding author at: Department of Physiology and Pharmacology, 3000 Arlington Avenue, Mailstop 1008, University of Toledo College of Medicine, Toledo, OH 43614, USA., Terry.Hinds@utoledo.edu (T.D. Hinds).

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ESRD includes hemodialysis, peritoneal dialysis, or kidney transplantation. Of the 660,000 Americans with ESRD, 468,000 require dialysis and >193,000 have a functioning kidney transplant. Studies have shown that kidney transplantation increases a patient's daily activities, and results in better survival rates [1]. However, there is also the possibility of organ rejection. In fact, according to the Organ Procurement and Transplantation Network, renal transplant failure rate within one and five years from a deceased donor is 4 and 21%, respectively, which decreases to 3 and 14% for live donors. Possibly, indicating that live donors have lower ischemia reperfusion injury in the living/related donor (LRD) causing lesser endothelial injury and possibly less coagulation of blood.

A factor in red blood cells (RBCs) is heme that sequesters oxygen, and during RBC breakdown, macrophages in the spleen, bone marrow, and liver engulf disrupted RBCs and separate the globin and heme portions from hemoglobin, myoglobin, and other heme-containing proteins. Heme is broken down by heme oxygenase (HO) to biliverdin which is converted to unconjugated bilirubin (UCB) by biliverdin reductase (BVR) [2–6]. Bilirubin is conjugated (CBR) in the liver by the glucuronosyltransferase enzyme UGT1A1 with glucuronic acid and secreted by multidrug resistant protein-2 (MRP2) through the canalicular membrane to bile in the gallbladder and eventually into the intestine (Fig. 1)[2, 7]. Damage to the liver can reduce conjugation causing high plasma UCB levels, and for this reason, bilirubin has long been associated with liver damage and jaundice. However, at micromolar concentrations in vitro, it is an efficient scavenger of peroxy radicals, acting as a potent antioxidant [8]. Additionally, UCB has been shown to be a robust immunomodulatory agent that dampens many of the pathways involved in transplant rejection [6, 9–13]. The immunomodulatory effect of bilirubin may be beneficial in organ transplantation [14], especially for kidney and heart as discussed further in [6]. Moreover, increased levels of UCB have been shown to be advantageous in various conditions caused by both oxidative stress and autoimmune diseases including pemphigus vulgaris [15]. Bilirubin may also play a role in vessel occlusion as it has been shown to prevent endothelial adhesion and angiotensin II-induced vasoconstriction [16, 17], and may have potential effects on platelet function [18]. Increased levels of UCB post-renal transplant have been shown to be a positive indicator of graft survival [19], which may be positively correlated with renal transplant acceptance. UCB reduces oxidative stress and inflammation that is increased in the obese which assists in the acceptance of kidney transplantation.

While immune hypersensitivity has long been a therapeutic target for organ acceptance, reducing oxidative stress and modulating the immune response via increasing bilirubin during renal transplantation may have therapeutic indications. Herein, we highlight key studies that demonstrate the significant roles of UCB and its impact on body weight and oxidative stress in the receipt of renal transplant.

1.1. Bilirubin and body weight in renal transplantation

Weight gain post-transplantation can have an adverse effect on transplant acceptance as shown by Hoogeveen et al. [20]. These authors concluded that an increase in BMI one-year post-transplant is more strongly related to death and graft failure than the pre-transplant BMI among kidney transplant recipients. An observational cohort study was performed by Kim et

al., which analyzed the relationship between post-transplant weight gain and kidney function regarding estimated glomerular filtration rate (eGFR) at 12 months [21]. The investigations showed that post-transplant weight gain within the first six months has a negative correlation with kidney function expressed in terms of eGFR [21]. They also revealed that 30% of the patients had a post-transplant weight gain, of which 27% gained >10% of their baseline weight. Additionally, in multivariate logistic regression analysis of 11,836 renal transplant recipients, Molnar et al. concluded that pre-transplant overweight/obesity was incrementally associated with an increased risk of delayed kidney graft function [22]. A meta-analysis by Lafranca et al. found that of the 56 studies they analyzed that there were significant benefits in renal allograft recipients with a BMI <30 in terms of mortality, delayed graft function, acute rejection and three-year survival rate [23]. The critical factor linking increased BMI and transplant rejection may be oxidative stress. A known phenomenon of obesity is elevated reactive oxygen species (ROS), which promotes inflammation and vascular disease [24]. UCB levels are decreased in the obese and increasing UCB lowers body weight and oxidative stress (Fig.2)[25].

Andersson et al. showed the relationship between weight loss and bilirubin levels as part of the Sibutramine Cardiovascular Outcome trial [26]. This study included a cohort of 10,198 patients, who met the inclusion criteria including weight measurements at the beginning and end of the four-week study period [26]. The authors concluded that there is a linear relationship between serum bilirubin levels and weight reduction. Each 1% increase in weight loss resulted in a rise in total bilirubin levels by 0.21 $\mu\text{mol/L}$ in men, and 0.11 $\mu\text{mol/L}$ in women [26]. Since obesity is linked with decreased success rates of renal allografts and bilirubin levels are inversely proportional to BMI, it is reasonable to hypothesize that exogenous bilirubin may lower the detrimental effects that obesity plays on renal allografts (Fig. 2). Hinds et al. showed that increasing heme oxygenase production of bilirubin in obese genetically engineered leptin receptor-deficient *ob/ob* mice reduced body weight and blood glucose [25]. Dong et al. assessed the impact of exogenous UCB on obesity, glucose metabolism, and inflammation in diet induced obese (DIO) and *ob/ob* mouse models [27]. It was noted that UCB-treated mice had a significant decrease in hyperglycemia, increased insulin sensitivity, and suppressed endoplasmic reticulum stress markers. Liu et al. later showed that UCB treatment in DIO C57Bl/6 mice reverses glucose and insulin intolerance and lowers plasma leptin levels [28], which controls appetite and is a known inflammatory factor [29]. Bilirubin has been shown to regulate the immune system by decreasing pro-inflammatory cytokine expression, including TNF- α , IL-1 β , and monocyte chemoattractant protein-1 [27]. A polyethylene glycol (PEG) modified bilirubin (PEGylated-bilirubin), which makes it more soluble, was shown to have anti-oxidative and anti-inflammatory properties and was beneficial in pancreatic islet xenotransplantation [30]. However, the PEGylated-bilirubin has not been used for any other applications. Bilirubin may be beneficial for the acceptance and long-term prognosis of renal allografts. However, more investigations are needed to improve our understanding of the protective role of bilirubin in weight management and renal transplant.

Lipid peroxidation during obesity contributes significant problems with allograft acceptance. In a 12-month pilot study of 33 renal transplant recipients, Cho et al. showed lipid peroxidation products thiobarbituric acid reactive substances (TBARS) were significantly

higher in the transplant recipients who gained weight compared to those who lost weight and recommended strategies to lower oxidative stress to aid in allograft acceptance [31]. Overall, studies on renal transplant recipients showed that weight gain and obesity cause increased oxidative stress which leads to transplant rejection. Since bilirubin has been shown to be a potent antioxidant, it may serve as a therapeutic for transplant, particularly in patients with an increased oxidative load due to excess BMI. The most common cause of hyperbilirubinemia in humans is a *UGT1A1*28* polymorphism known as Gilbert's Syndrome (GS) (Fig. 1) [32]. Crigler-Najjar is a more extreme form of hyperbilirubinemia caused by total or partial deficiency of the UGT enzyme due to a mutation in the five exons of *UGT1A1* [33]. The GS polymorphism, *UGT1A1*28*, which contains an additional TA repeat in the TATA sequence of the *UGT1A1* promoter reduces expression resulting in slightly higher (50–100%) plasma unconjugated BR levels [34, 35]. Interestingly, patients exhibiting mildly elevated BR levels were also shown to have significantly less metabolic disorders such as nonalcoholic fatty liver disease (NAFLD), obesity or type II diabetes [36–41]. In a humanized mouse model for GS (hGS mice) that contains the human *UGT1A1*28* polymorphism also displayed unconjugated hyperbilirubinemia [42], and on a high-fat diet, had decreased lipid accumulation and resistance to hepatic steatosis [42]. Interestingly, the hGS mice had significantly increased the activity of the lipid-reducing transcription factor peroxisome *proliferator-activated receptor-α* (PPARα) [42]. Molzer et al. conducted a study with GS patients and reported similar increases in PPARα expression [43]. Bilirubin was shown to activate PPARα directly [44]. PPARα has been shown to prevent high-fat diet-induced renal cell apoptosis and oxidative stress in spontaneously hypertensive rats [45], as well as plays a crucial role in L-carnitine anti-apoptosis in renal tubular cells [46]. The effect of bilirubin on PPARα in the kidney is not known, especially its role in the acceptance of renal transplantation.

1.2. Bilirubin in renal hemodynamics

Many factors contribute to the decline in renal blood flow following transplantation, such as damage to the vascular endothelium causing thrombosis, and increased levels of vasoconstrictors including angiotensin II and endothelin [47, 48]. Additionally, the effects of calcineurin inhibitors (CNI's) and immunosuppressive drugs such as cyclosporine and tacrolimus also reduce renal blood flow [41–43]. These agents reduce renal blood flow through their effects on vasoconstrictors such as angiotensin II, endothelin, 20-HETE, and thromboxane as well as by inhibition of nitric oxide (NO) production [49–51]. Furthermore, CNIs lower renal blood flow through enhanced production of ROS [52, 53]. UCB has been shown to have beneficial effects on the vascular endothelium as well as oppose endogenous vasoconstrictor molecules. Mazzone et al. showed that UCB had an inhibitory effect on polymorphonuclear cells (PMNCs) and endothelial adhesion, which was beneficial on atherosclerotic disease [16]. The potential for UCB to protect from endothelial dysfunction after renal transplant should be evaluated as it has been shown to be preventative in CVD.

Numerous studies have examined the effects of increased levels of plasma bilirubin on the pressor response to angiotensin II. The severely hyperbilirubinemic Gunn rat exhibited resistance to angiotensin II-induced vasoconstriction [49]. Also, mice made moderately hyperbilirubinemic via partial knockdown of hepatic *Ugt1a1*, exhibited improvements in

glomerular filtration rate, renal blood flow, and renal vascular resistance in angiotensin II-dependent hypertension (Fig. 1) [54]. This improvement in renal hemodynamics by moderate hyperbilirubinemia was associated with an increase in the bioavailability of NO, independent of any specific changes in the levels of nitric oxide synthase 3 (NOS3) [55]. The ability of UCB to increase the bioavailability of NO was due in part to its antioxidant actions. NO reacts with superoxide anion (O_2^-) to form peroxynitrite, a potent oxidant [6]. Furthermore, bilirubin reduces cellular production of O_2^- by direct scavenging as well as by inhibition of NAD(P)H oxidase enzymes responsible for the O_2^- generation to lower peroxynitrite formation [8, 56, 57]. Angiotensin II has also been shown to increase vascular O_2^- production, which contributes to the vasoconstrictor actions of the peptide [58–61]. Thus, bilirubin diminishes the pressor response to angiotensin II in part through lowering of vascular O_2^- production. Unconjugated bilirubin has also been reported to affect NO levels through regulation of NOS3 phosphorylation, although this has not been a universal finding [55, 62, 63].

The ability of bilirubin to improve renal blood flow, renal vascular resistance, and glomerular filtration rate has traditionally been thought to be mediated by its antioxidant actions; however, several studies suggest that bilirubin may alter vascular function through a new mechanism. There may be significant differences between UCB and CBR on signaling and how they may affect the kidney. Total bilirubin was negatively associated with urine protein and positively linked with eGFR in Korean adults [64]. Others have shown that total bilirubin levels are associated with mortality in uremia patients undergoing long-term dialysis [65]. These studies did not, however, consider the amount of CBR or UCB present, which is critical as only UCB has an effect on reducing inflammation and obesity. UCB was recently demonstrated to be an activator of nuclear receptors, in particular, PPAR α [4, 44, 66]. *The discovery of this novel action of UCB was intriguing given the fact that a recent study reported that the antioxidant activity of bilirubin only accounted for a fraction of the antihypertensive effects of moderate hyperbilirubinemia in a model of angiotensin II-dependent hypertension* [67]. *Previous studies have demonstrated the protective effects of PPAR α induction on renal hemodynamics in different models of renal insufficiency* [68, 69]. *It is possible that UCB acting through PPAR α could preserve renal blood flow following transplant via an increase in vasodilatory compounds such as epoxyeicosatrienoic acids (EETs) or NO* [70].

Transplant rejection can be induced by cross-reactivity of antigens leading to a hypersensitized immune response and ischemia and reperfusion (IR) injuries. IR injuries are due to the lack of blood supply after organ harvest, and the subsequent recirculation of the blood after a successful transplant. During ischemic injury, lack of oxygen causes a buildup of metabolic wastes along with the depletion of ATP [71, 72]. Oxidative damage occurs because of an increase in ROS as seen with reperfusion injury as well as a disruption between the balance of oxidants and antioxidants [73]. During reperfusion, rewarming, reoxygenation, and return to aerobic metabolism allows for the already occurring damage to be exacerbated by inflammation, which includes the release of cytokines, chemokines, and

complements resulting in activation and migration of leukocytes [71, 74]. Antioxidants work by counteracting the harmful effects of ROS, but the rapid production of oxidative stress as seen in kidney transplantation can overwhelm the oxidant load leading to injury and cell death [75]. Ultimately, these injuries result in reduced renal blood flow and tissue damage and death.

1.3. Bilirubin effects on oxidative stress and renal transplant

Bilirubin is a potent antioxidant which can reduce inflammation. An investigation by Boon et al. determined the antioxidant effects of UCB by measuring lipid and protein oxidation in hyperbilirubinemic humans and rats [76]. They analyzed blood/serum sample susceptibility to hypochlorous acid (HOCl), and a combination of myeloperoxidase (MPO), hydrogen peroxide (H₂O₂) and Cl⁻, which are known to oxidize proteins and lipids [76]. The blood/serum samples were either infused with exogenous UCB (<250 μM) or were from species with increased endogenous UCB, which included Gunn Rats and patients with Gilbert's syndrome (GS) polymorphism (UGT1A1*28) [76]. The human trial contained 21 patients with GS vs. 21 control patients, and the animal trial included 9 Gunn rats with 5 control specimens [76]. Boon et al. concluded that patients with GS, as well as Gunn rats, had significantly reduced levels of lipid and protein oxidation compared to controls. Additionally, exogenous UCB reduced levels of protein and lipid peroxidation in a dose-dependent manner [76]. In a rodent model of adenine induced renal failure, circulating markers of oxidative damage were improved in hyperbilirubinemic Gunn rats as well as the effects of adenine-induced tubule-interstitial injury [77].

Several markers are used as indicators of post-transplant oxidative stress, including malondialdehyde (MDA), which has been shown to be a predictor of kidney graft dysfunction [78]. A prospective study by Fonseca et al. analyzed 40 kidney transplant recipients and concluded that the postoperative mean MDA levels were significantly higher in patients with delayed graft function [78]. A study of 108 patients with GS without cardiovascular risk factors along with 108 matched controls concluded that biomarkers of oxidative stress, including serum concentration of MDA-modified low-density lipoprotein and urinary excretion of 8-hydroxy-2-deoxyguanosine, were significantly lower in patients with GS [79]. Other studies have also considered patients with GS who have received a renal transplant to determine whether the increased bilirubin levels were promising for graft acceptance [19]. Lee et al. analyzed 429 renal transplant recipients, 118 of whom experienced biopsy-proven acute rejection (BPAR). Among this group, it was determined that although pre-transplant bilirubin levels did not affect the development of rejection, 1-year post-transplant levels of those who did not experience BPAR were significantly higher than those who did [19]. Furthermore, it was determined that the UGT1A1*28 as seen in those with GS was a protective factor against renal transplant rejection [19].

The use of exogenous bilirubin has also been studied in animal models. Adin et al. [80] experimented using mice to test the effects of exogenous bilirubin administration on renal transplant acceptance. In this study, a bilirubin flush was administered 20 min before warm ischemia, and the results showed significant improvements in renal vascular resistance, urine output, glomerular filtration rate, tubular function, and mitochondrial injury after ischemia-

reperfusion injury [80]. A prospective study looking at renal transplant recipients with a functioning graft for over a year concluded that circulating levels of bilirubin were inversely correlated with late graft failure [81]. From these studies, we suggest that exogenous bilirubin could help reduce ischemia-reperfusion injuries due to oxidative stress, which will in turn help with renal transplant acceptance (Fig. 1).

1.4. Bilirubin's effects on inflammation

Bilirubin has been shown to reduce inflammatory pathways, and this may be beneficial for kidney transplantation (Fig. 2). NO is an integral part of acute and chronic inflammatory diseases and an essential component of ischemia reperfusion injury especially in transplantation [82]. Using a murine model to study endotoxemia, Wang et al. determined that bilirubin inhibits the upregulation of LPS inducible nitric oxide synthase (iNOS), which catalyzes the production of NO from L-arginine by suppressing TLR-4 [83]. They concluded that there might be a role for bilirubin in reducing tissue injury in response to inflammatory stimuli [83]. This idea lends the potential for bilirubin to act as a mediator to reduce tissue injury from inflammation post renal transplantation.

Several studies have shown a negative relationship between bilirubin and serum amyloid A (SAA), which has been shown to be a marker of kidney transplant rejection. C-reactive protein (CRP) and SAA have long been known to be biomarkers of inflammation that are negatively linked to renal transplant acceptance [84–86]. A study regarding SAA and kidney rejection was performed by Maury et al. who showed that 10 renal transplant recipients had significantly elevated SAA levels in all cases of rejection [87]. In patients with lower bilirubin, SAA levels have been shown to be higher [88]. In people with Gilbert's syndrome that are over 30 years of age have significantly reduced SAA levels 3.61 mg/L compared to 4.75 mg/L in age-matched controls [89]. Interestingly, in people with GS and control <30 years of age had similar levels of SAA levels at 3.42 mg/L for GS and 3.47 mg/L for control [89]. However, in this study, the BMI of the patients were low at 22.6 for GS and 22.1 for control under 30 and 26.0 for GS and 24.2 for control over 30, which might account for the lower levels of SAA.

Bilirubin plasma levels are lower in patients with obesity [5, 25, 27, 28, 44, 66, 90, 91], and SAA and CRP levels are higher [88]. A study monitoring SAA via micro-ELISA (micro enzyme-linked immunosorbent assay) to detect early kidney rejection found that 20 of 22 rejection episodes (91%) showed that SAA elevation predicted rejection, and rose sharply two days prior [86]. The authors concluded that SAA was a better predictor of rejection than CRP and should be monitored every day in patients with a kidney allograft [86]. A study conducted by Fukuda et al. had found similar results and conclusion [84]. They looked at two renal transplant recipients with acute graft failure and found that while SAA was elevated on the sixteenth day, CRP showed no change [84]. The studies proposed that lower levels of SAA may be beneficial in kidney transplantation. Importantly, bilirubin has been shown to be negatively associated with SAA levels in obese [88] or older patients [89], which suggest that elevated UCB is potentially beneficial for kidney transplant in these patient populations. Notably, bilirubin levels have been shown to be inversely proportional to levels of SAA in those without metabolic syndrome [88]. A similar result was demonstrated by

Wallner et al. using a hyperbilirubinemic Gunn rat model which showed significantly lower levels of proinflammatory cytokines, including SAA, concluding that elevated bilirubin is associated with reduced levels of inflammatory biomarkers [92]. Extrapolation of data from studies showing SAA as a marker of kidney rejection and studies demonstrating the inverse relationship between bilirubin and SAA suggests that elevated UCB may potentially be beneficial for a kidney transplant acceptance.

A limitation of the human study by Deetman et al. demonstrated an inverse relationship between SAA and bilirubin in a rather small population size of 167 subjects. Moreover, the relationship was only noted among those without metabolic syndrome and was not present in those with coexisting metabolic syndrome. More human studies evaluating the relationship between SAA and bilirubin will help to solidify or disprove these conclusions.

Evovquoz et al. measured CRP daily for 28 days following renal transplantation of 45 patients. They determined that even under aggressive immunosuppression, CRP can aid in the early diagnosis of graft rejection following renal transplantation [85]. Ozdemir et al. had similar results, noting a high negative predictive value for renal allograft survival in patients with consistently elevated serum CRP [93]. Interestingly, a cross-sectional study of 2307 Korean adults, showed that CRP levels tended to decline as total and indirect bilirubin levels increased [69]. Petronella et al. compared 94 subjects with the metabolic syndrome (MetS) with 73 control subjects and determined that in all subjects CRP was inversely related bilirubin levels [88]. Additionally, they found that bilirubin was lower in patients with MetS, coinciding with increased levels of CRP and SAA [88].

The above studies lend sufficient evidence to suggest that higher levels of bilirubin may reduce biomarkers commonly associated with renal transplant rejection, namely SAA and CRP, as elevated UCB has been shown to lower both SAA and CRP levels. Further studies on bilirubin are needed to determine its benefits in kidney transplant.

1.5. Bilirubin as an immunomodulatory agent for kidney transplant

In order to combat a hypersensitized immune system, immunosuppressive drugs are typically required during kidney transplantation. These include various medications that dampen T-cell activation by either blocking signal 1, the interaction between the T-cell receptor complex and antigen presenting cell, the co-stimulatory signal, CD80/ 86 on the antigen presenting cell (APC) with CD28 on the T cell, or the downstream signals after activation (Fig. 3). Immunosuppressant's for renal transplant include calcineurin inhibitors (CNIs) such as cyclosporine and tacrolimus [94]. UCB has immunomodulatory activities that reduce effector T cell responses, interception of the complement cascade, and promotion of regulatory T cell expansion (discussed further in [95]), all of which, may be beneficial in kidney transplant acceptance (Fig. 2).

Liu et al. determined that bilirubin has a unique effect of modulating the immune response of mice that lack other antioxidants [9]. This study used exogenous bilirubin on SJL/J mice and concluded that bilirubin suppressed CD4⁺ T cell response at several steps [9]. Bilirubin treatment resulted in the dampening of CD4⁺ T cell response via inhibition of co-stimulatory actives, suppression of immune transcription factor activation, and downregulation of

MHCII expression [9]. The immunomodulatory effects of bilirubin were also shown by Haga et al. who determined that interleukin 2 (IL-2) production by human peripheral blood mononuclear cells (PBMNC) was significantly lower after bilirubin treatment (Fig. 3)[10]. This is particularly important because IL-2 is a biomarker for kidney rejection [11–13]. UCB may have protolerogenic properties that allow for acceptance of the tissue [96, 97]. Heme oxygenase production of UCB has been shown to induce tolerance in patients with islet allografts by modulating T regulatory cells [98]. Furthermore, Rocuts et al. used mouse models to demonstrate that UCB treatment promotes de novo synthesis of regulatory T cells [97]. Additionally, transfer of T-regulatory cells resulted in long-term acceptance skin allografts [97]. They concluded that regulatory T-cells are necessary for the tolerance induction of treatment with UCB [97]. Studies utilizing genetic loss and gain functions have shown that IL-2 receptor activation is essential for regulatory T cell function and suppressor activity (Fig. 3) [99]. This study further went on to demonstrate that T regulatory suppressor activity following IL-2R activation was mediated by production of the transcription factor STAT5 and IL-2R deficient regulatory T cells could restore function by introducing STAT5 [99]. Bilirubin treatment reduces IL-2 levels, and STAT5 introduction may be necessary for transplant acceptance.

The effect of bilirubin on modulating the immune system may have several beneficial advantages. Mazzone et al. demonstrated that HUVEC and H5V cells treated with bilirubin inhibited TNF α related induction endothelial adhesion molecules, which may translate to protection against cardiovascular disease [16]. Dong et al. also noted that increased bilirubin levels were associated with decreased inflammatory cytokines, including TNF α [27]. The effects of bilirubin treatment on TNF α were shown in a study of six rejected kidney transplants, using in-situ hybridization of TNF α mRNA showed high expression in monomorphic infiltrating cells in the deep cortex and around the cortical collecting tubes [100]. Sonkar et al. further analyzed TNF α levels in 29 renal transplant cases compared with 20 healthy controls and 21 hemodialysis patients [101]. They determined that TNF α in healthy controls ranged from 2 to 15 pg/mL, whereas in patients with chronic renal failure and renal transplant rejection, TNF α levels were above 45 pg/mL [101]. In comparison, those with stable transplants had a TNF α level between 16 and 30 pg/mL [101]. They concluded that a TNF α level of >45 pg/mL could be an immunological marker for transplant rejection [101]. When plasma samples were collected from a sample of 25 renal transplant recipients, it was determined that when observing cases of biopsy-confirmed acute rejection, TNF α levels were significantly increased [102]. Further studies should be conducted to determine if bilirubin's antagonism of TNF α may play a beneficial role in renal transplantation rejection.

2. Conclusion

Patients with elevated UCB have decreased pro-inflammatory cytokines, and increased antioxidant capacity, all of which have been shown to be protective against renal transplant rejection. Also, there has been at least one human study concluding that patients with hyperbilirubinemia due to GS and elevated post-transplant levels of bilirubin could predict good outcomes of renal transplant acceptance. Finally, there have been several studies showing that successful kidney transplant recipients had elevated bilirubin levels

postoperatively. Therefore, further studies need to be conducted on patients receiving kidney transplants to determine the role of bilirubin in allograft tolerance and acceptance. Importantly, UCB may serve as a novel therapy for protection of kidney transplantation, or regulation of enzymes (biliverdin reductase and UGT1A1) that control plasma levels.

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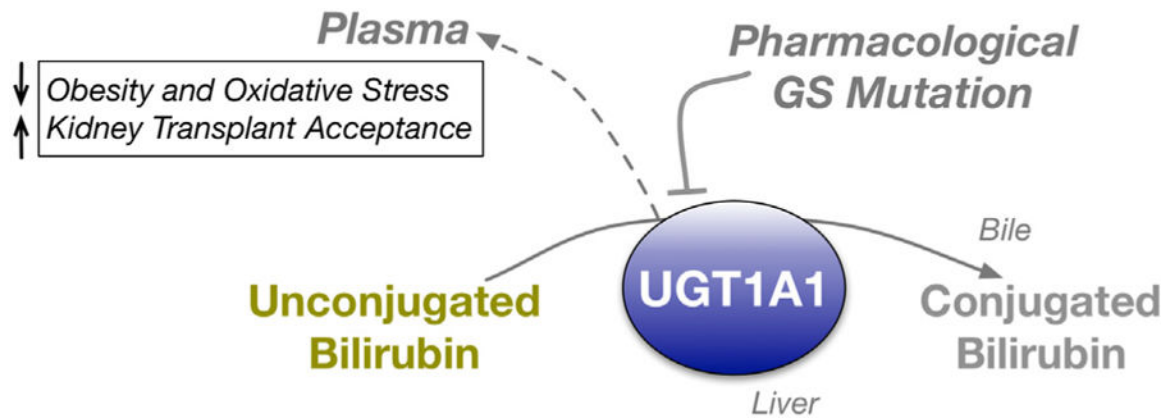


Fig. 1.

Regulation of plasma bilirubin levels increases renal transplant acceptance. Unconjugated bilirubin is conjugated by the UDG-glucuronyltransferase enzyme UGT1A1 and deposited into bile. Decreasing activity of UGT1A1, as with the Gilbert's polymorphism (GS) or by pharmacological inhibition, results in hyperbilirubinemia. Increasing plasma unconjugated bilirubin levels reduces obesity and oxidative stress and increases the likelihood of kidney transplant acceptance.

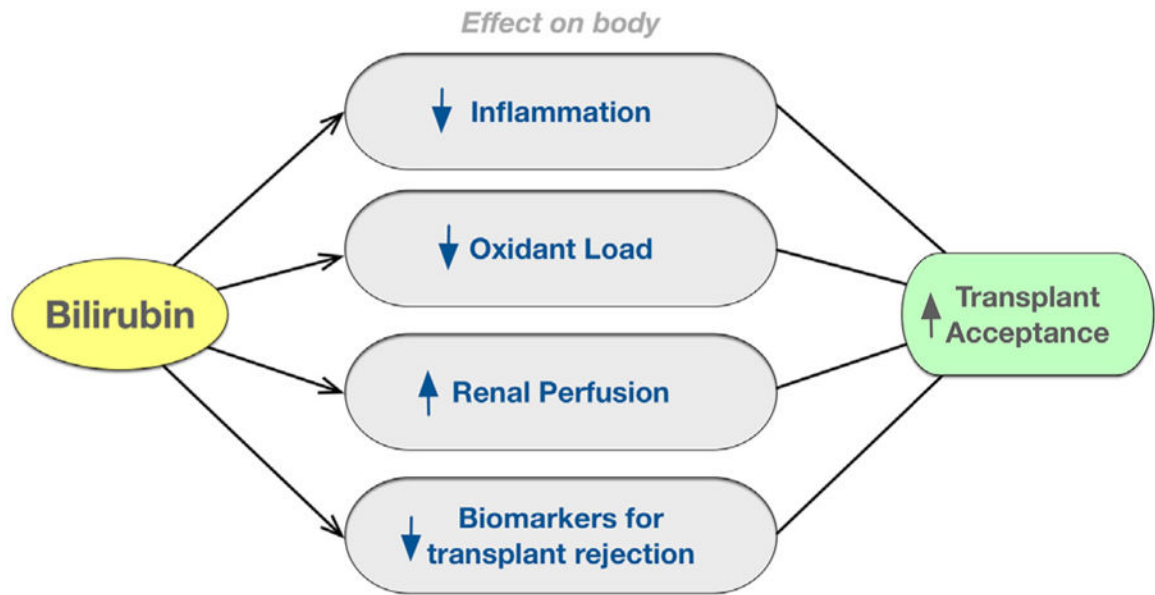


Fig. 2.

Systemic effects of bilirubin may aid in transplant acceptance. Bilirubin has been shown to have several systemic effects on the body including a decrease in inflammatory markers, oxidant load, and biomarkers for transplant rejection as well as an increase in renal perfusion.

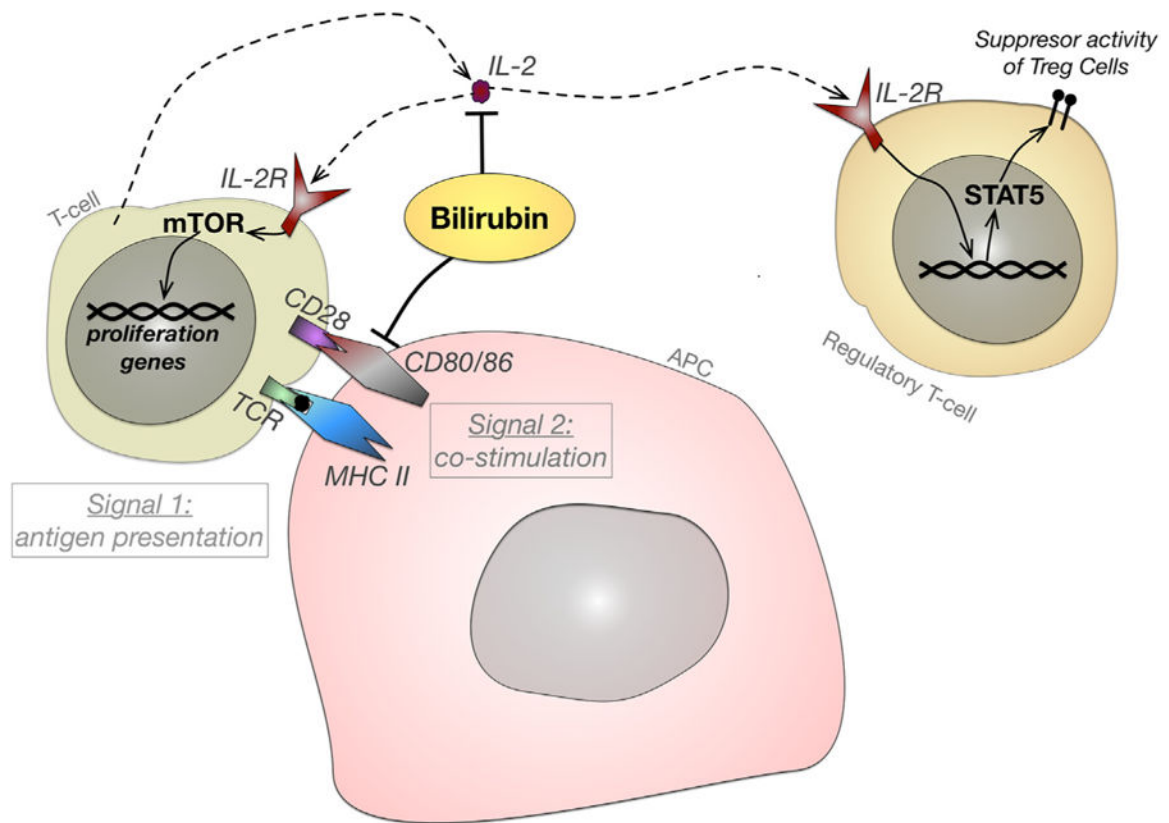


Fig. 3.

Immunomodulatory effects of bilirubin. Antigen mediated T-cell activation requires two signals. Signal one is antigen presentation via MHCII on antigen presenting cells (APC) to the T cell receptor (TCR). Signal 2 is CD80/86 activation on the APC with CD28 on the T cell. Activated T cells produce interleukin-2 (IL-2) which activates the IL-2 receptor (IL-2R) on T cells and regulatory T-cells. Bilirubin reduces IL-2 levels. IL-2R activation increases regulatory T cell suppressor activity. Suppressor activity is mediated by the transcription factor, STAT5 following IL-2R activation of STAT5 for suppressor effects.