ORIGINAL ARTICLE

Suppression of human alloreactive T cells by linear tetrapyrroles; relevance for transplantation

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The main limitation to successful transplantation is the antigraft response developed by the recipient immune system, and the adverse side effects of immunosuppressive agents which are associated with significant toxicity and counter indications such as infection and cancer. Furthermore, immunosuppressants do little to prevent ischemia-reperfusion injury during the transplantation procedure itself hence there is a growing need to develop novel immunosuppressive drugs specifically aimed at prolonging graft survival. Linear tetrapyrroles derived from the breakdown of mammalian heme have been shown in numerous studies to play a protective role in allograft transplantation and ischemia-reperfusion injury; however, commercial sources of these products have not been approved for use in humans. Plants and algae produce equivalent linear tetrapyrroles called bilins that serve as chromophores in light-sensing. One such marine-derived tetrapyrrole, phycocyanobilin (PCB), shows significant structural similarity to mammalian biliverdin (BV) and may prove to be a safer alternative for use in the clinic if it can exert direct effects on human immune cells. Using a mixed lymphocyte reaction, we quantified the allogeneic responses of recipient cells to donor cells and found that PCB, like BV, effectively suppressed proliferation and proinflammatory cytokine production. In addition, we found that BV and PCB can directly downregulate the proinflammatory responses of both innate dendritic cells and adaptive T cells. We therefore propose that PCB may be an effective therapeutic drug in the clinical setting of transplantation and may also have wider applications in regulating inappropriate inflammation. (Translational Research 2016;■:1–14)

Abbreviations: HO-1 = heme oxygenase; BV = biliverdin; PCB = phycocyanobilin

INTRODUCTION

Immunosuppressive therapy, although necessary after transplantation, is associated with many adverse consequences, including increased susceptibility to infection and cancer. Furthermore, immunosuppressants do little to prevent ischemia-reperfusion injury (IRI) or chronic rejection, and there has been a gap in the market over the last decade in the generation of

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AT A GLANCE COMMENTARY

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Background

Patients receiving allografts require life-long immunosuppression which is often associated with increased risk of infection and cancer, hence, there is a need to develop less toxic treatment regimens specifically aimed at prolonging graft survival. Linear tetrapyrroles derived from the breakdown of mammalian heme play a protective role in allograft transplantation/ischemia-reperfusion injury however commercial sources of these products are not fit for human use.

Translational Significance

We demonstrate that a plant/algae derived tetrapyrrole, phycocyanobilin, significantly attenuates harmful pro-inflammatory responses to allogeneic cells and propose that it may be an effective therapeutic in the clinical setting of transplantation and dysregulated inflammation.

new therapies with safer side-effect profiles aimed at prolonging graft survival and combating oxidative damage. Several groups have reported that induction of the stress response protein, heme oxygenase-1 (HO-1), improves outcome after experimental transplantation and have attributed this protective effect to the heme breakdown products, carbon monoxide (CO), and the tetrapyrroles, biliverdin (BV) and bilirubin.[1-6] Administration of BV in a mouse model of heart transplantation significantly prolonged graft survival, resulted in enhanced tolerance to donor antigens and was accompanied by diminished immune cell infiltration in the grafts themselves.[7] Furthermore, mesobiliverdin, a synthetic analogue of the seaweed–derived linear tetrapyrrole, phycocyanobilin (PCB), was shown to enhance pancreatic islet yield and function in a model of islet transplantation which is currently being explored as a treatment option for type 1 diabetes.[2] In addition to promoting tolerance, induction of HO-1 expression and administration of BV or bilirubin have also been reported to protect in models of IRI.[1-6] For example, in a model of liver transplantation, after 16 hours of cold ischemia, rinse of the liver graft with bilirubin before reperfusion improved survival rate from 67% to 100%.[8] Furthermore, adding BV to the preservation and perfusate solution itself, significantly improved graft function following transplantation.[4] The protective effects of BV and bilirubin are attributed to their ability to downregulate the production of proinflammatory cytokines from innate immune cells which in turn modulate adaptive T cell responses—the target of most immunosuppressive agents. In addition, BV and bilirubin are powerful antioxidants which most likely accounts for their protective effects during reperfusion when reactive oxygen species are known to be elevated.

Given the established immunomodulatory effects of linear tetrapyrroles in various animal models of disease, studies examining their effects on human immune cells are surprisingly few. Furthermore, little is known regarding the disease-ameliorating effects of plant/algae-derived linear tetrapyrroles such as PCB. A recent study has, however, demonstrated that phycocyanin, which contains the active PCB moiety, has potent radical scavenging activity in human leukocytes and effectively reduces glucose oxidase-induced inflammation in the mouse paw suggesting that immunomodulatory activity is present.[9] Commercially available BV, whether synthetic or extracted from bovine bile, has not been approved for human use and large scale production of BV in *E.coli* or yeast expression systems has been attempted but is hindered by potential endotoxin contamination. Therefore, plant/algae-derived tetrapyrroles such as PCB may prove to be a safer alternative for use in the clinic assuming they can exert direct effects on human immune cells.

Using an *in vitro* model of human allograft responses, we assessed the ability of BV and PCB to downregulate the harmful proinflammatory alloresponse of recipient peripheral blood mononuclear cells (PBMCs) to donor PBMC. In addition, we sought to determine the immune cell types through which these linear tetrapyrroles exert their effects. We found that BV and PCB significantly attenuated the harmful proinflammatory response to allogeneic cells and furthermore show that BV and PCB can exert direct effects on innate antigen presenting dendritic cells (DCs) and adaptive T cells, which both play a prominent role in chronic rejection post organ transplantation.

MATERIALS AND METHODS

Human blood samples. This study was approved by the research ethics committee of the School of Biochemistry and Immunology, Trinity College Dublin and is in accordance with the Declaration of Helsinki. Leukocyte-enriched buffy coats from anonymous healthy donors were obtained with permission from the Irish Blood Transfusion Service, St. James's Hospital, Dublin. PBMC were isolated by density gradient centrifugation (Lymphoprep; Axis-Shield poC). Cells were cultured in RPMI medium (Biosera)