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Bilirubin acts as an endogenous regulator of inflammation by disrupting adhesion molecule-mediated leukocyte migration

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Abstract

There is a growing body of evidence that bilirubin, which is generated during the physiological breakdown of heme, exerts potent anti-inflammatory effects. Previous work by our group suggests that bilirubin is able to suppress inflammatory responses by preventing the migration of leukocytes into target tissues through disruption of vascular cell adhesion molecule-1 (VCAM-1)-dependent cell signaling. As VCAM-1 is an important mediator of tissue injury in the dextran sodium sulfate (DSS) murine model of inflammatory colitis, we examined whether bilirubin prevents colonic injury in DSS-treated mice. As anticipated, bilirubin-treated animals manifested significantly less colonic injury and reduced infiltration of inflammatory cells into colon tissues. We further observed that bilirubin administration was associated with a reduced number of eosinophils and monocytes in the small intestine, with a corresponding increase in peripheral blood eosinophilia, regardless of whether mice received DSS. These findings suggest that bilirubin impairs the normal migration of eosinophils into intestinal tissues, as supported by *in vitro* experiments showing that bilirubin blocks the VCAM-1-dependent movement of Jurkat cells across human endothelial cell monolayers. Taken together, our findings support that bilirubin ameliorates DSS-induced colitis and disrupts the physiological trafficking of leukocytes to the intestine by preventing transmigration across the vascular endothelium, potentially through the inhibition VCAM-1-mediated signaling. Our findings raise the possibility that bilirubin functions as an endogenous regulator of inflammatory responses.

Keywords

bilirubin; VCAM-1; eosinophil; DSS colitis; endothelium

In all mammalian species, bilirubin is generated during the normal physiological breakdown of heme through the sequential activity of the heme oxygenase and biliverdin reductase enzymes. It was first postulated over 75 years ago that bilirubin exerts anti-inflammatory effects when it was noted that patients with rheumatoid arthritis experienced remission after developing jaundice from superimposed liver disease [1, 2]. More recent epidemiological studies have correlated increased serum bilirubin levels with a decreased incidence of

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inflammatory conditions such as asthma [3], multiple sclerosis [4], and Crohn's disease [5]. Experimentally, bilirubin has been shown to ameliorate tissue injury in murine models of allergen-induced inflammation, including experimental autoimmune encephalomyelitis [6] and allergic pneumonitis [7]. In the latter study, our group demonstrated that the administration of bilirubin via intraperitoneal injection markedly suppressed allergen-induced pulmonary inflammation in immunoprimed mice, principally by preventing VCAM-1-mediated eosinophil infiltration into the lung. At the cellular level, we have further demonstrated that bilirubin, a potent chain-breaking antioxidant [8, 9], inhibits VCAM-1-dependent migration of leukocytes across endothelial monolayers by scavenging NADPH oxidase-generated reactive oxygen species [7], which are key intermediaries of endothelial cell retraction [10].

VCAM-1-mediated infiltration of eosinophils into the intestinal mucosa has been implicated in the pathogenesis of both ulcerative colitis and Crohn's disease [11-13], and it has been shown that treatment of rodents with antibodies [14-16] or antisense oligonucleotides [17] directed against VCAM-1 suppresses intestinal inflammation in colitis models. In humans, blocking antibodies directed against leukocyte integrins that bind VCAM-1 have been demonstrated to ameliorate Crohn's disease [18, 19]. Based on our previous work indicating that bilirubin is able to disrupt VCAM-1-mediated endothelial cell signaling, we speculated that treating mice with this bile pigment would prevent colitis induced by DSS, which is a VCAM-1-dependent process [16], by preventing the migration of inflammatory cells across vascular endothelium. In the present study [20], we found that DSS-treated mice that are simultaneously administered i.p. bilirubin experience markedly diminished disease severity as compared with vehicle-treated animals. Concordant histopathological analyses revealed that bilirubin-treated mice manifested significantly less colonic injury in response to DSS, with a marked reduction in the infiltration of eosinophils, lymphocytes, and monocytes into the colon. As bilirubin previously has been shown not to alter the production of pro-inflammatory cytokines and chemokines [7], these findings support the hypothesis that bilirubin suppresses DSS-induced intestinal injury by inhibiting VCAM-1-mediated leukocyte migration into the colon.

We further observed that mice receiving bilirubin demonstrated reduced numbers of eosinophils in the small intestine, even in the absence of DSS treatment [20]. As eosinophils are primarily tissue resident cells [21], these data indicate that, in addition to preventing leukocyte recruitment in response to inflammatory stimuli, bilirubin also inhibits the normal physiological trafficking of eosinophils to the gastrointestinal tract. This conclusion is supported by the coincident increase in peripheral blood eosinophilia detected in bilirubin-treated animals. We further noted that bilirubin administration was associated with reduced levels of monocytes in the small intestine, while the number of T-lymphocytes remained unaltered [20]. These findings suggest that eosinophils and monocytes may share a similar mechanism of recruitment to the small bowel. At the cellular level, we also demonstrated that bilirubin, at physiological concentrations (≤ 20 μM), prevents the migration of Jurkat cells (a T-cell leukemia line) across monolayers of human umbilical vein endothelial cells [20]. Since this process has been shown to be predominantly VCAM-1-mediated [22, 23], these data provide additional support for the proposition that bilirubin impedes leukocyte homing to target tissues by disrupting endothelial VCAM-1 signaling.

As VCAM-1 is an important mediator of leukocyte recruitment that occurs in response to certain infections and other inflammatory conditions [10], it stands to reason that bilirubin may act to attenuate these disorders. In support of this hypothesis are epidemiological analyses that have correlated increased serum bilirubin levels with a decreased incidence of certain disorders in which VCAM-1 has been shown to play a pathogenic role, including asthma [3], multiple sclerosis [4], and coronary artery disease [24, 25]. Our investigations support a mechanism of bilirubin action that involves the inhibition of VCAM-1-dependent leukocyte translocation across vascular endothelia through the scavenging of reactive oxygen species signaling intermediaries (Figure 1). We speculate that bilirubin, produced by the action of heme oxygenase-1 (which is induced in response to pro-inflammatory stimuli), serves as an endogenous regulator of host inflammatory responses.

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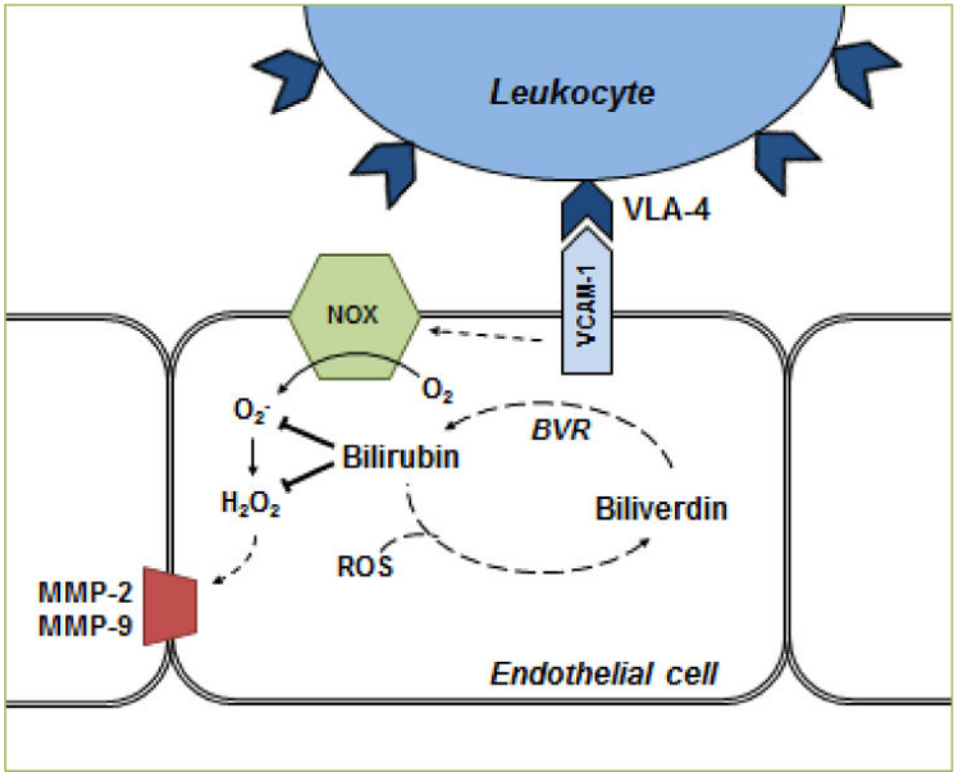

Figure 1. Proposed mechanism of bilirubin modulation of VCAM-1-dependent leukocyte migration

As originally delineated by Cook-Mills *et al*. [10], the binding of leukocyte VLA4 to endothelial VCAM-1 triggers the calcium- and Rac1-dependent activation of NADPH oxidase (NOX), resulting in the production of superoxide (O_2^-) and hydrogen peroxide (H_2O_2). These reactive oxygen species (ROS) stimulate downstream activation of matrix metalloproteinases (MMP)-2 and -9, which leads to the disruption of endothelial tight junctions and facilitates leukocyte transmigration. Bilirubin, a membrane permeant [26] and highly potent chain-breaking antioxidant [9] that undergoes intracellular redox cycling (dotted lines) through the action of bilirubin reductase (BVR) [8], scavenges NOX-derived ROS signaling intermediaries [7, 27], thereby inhibiting endothelial retraction and leukocyte migration.