Myeloperoxidase: a potential target of cyclic nitroxide(s) to ameliorate colitis ...

Environment, Animals

Inflammatory bowl diseases (IBD; Crohn’s disease; ulcerative colitis) are a group of inflammatory disorders with idiopathic etiology. These are debilitating conditions and affected patients experience abdominal pain, vomiting, diarrhea, fever, rectal bleeding and weight loss (1). Malabsorption and blood loss can lead to nutritional deficiencies and anemia; extra intestinal manifestations include musculoskeletal pain, retarded growth, liver disease, urologic complications, inflammation of joints and eyes (2, 3). Due to idiopathic etiology there is no permanent cure and the symptoms are managed through immunosuppressant, non steroidal anti-inflammatory (NSAID) drugs, and dietary changes to minimize environmental triggers and in severe cases surgical resection of the damaged bowl.

However, NSAIDs are contraindicated and long term steroid/immune therapy is limited. The peak manifestation of the disease starts as early as in 20s to mid-60s and affects both genders equally. Incidence rates of IBD are rising in younger population; Australian IBD patients tend to retire early and A$3. 1 billion (4) productive loss has been attributed to IBD with an estimate of ~ 100, 000 new cases to be reported by 2022 (5).

Cost associated with future IBD management and treatments is likely underestimated and development of new IBD treatment/therapies is direly needed. Current conventional treatment by NSAID is contraindicated in gastrointestinal disorders and long term use of corticosteroids exhibit sides effects (6-8). Therefore establishing an innovative, non-immunological therapy will alleviate the inflammatory course of IBD and thus minimize our dependency on immunosuppressant drugs which may have potential side

effects. Central to Pathophysiology of IBD is the progressive bowel inflammation induced by a cell mediated response where neutrophils and monocytes are the primary immune cells infiltrating the bowl and featuring IBD. These immune cells uniquely secrete the heme myeloperoxidase (MPO) enzyme that produces strong two-electron halogenated oxidant including hypochlorous acid (HOCL). MPO accounts 5 and 1. 65% of dry mass of neutrophils and monocytes respectively (9, 10).

Primarily, nutrophils and to lesser extent monocytes generate pathological amount of reactive oxygen species (ROS) during phagocytic and biocidal processes via MPO halogenation pathway. The biocidal action of neutrophil-MPO comes though halogenations cycle generating hypohalous acids including HOCL (11). Elevated levels of MPO and 3-chloro-tyrosine (3-Cl-Tyr; specific marker for HOCL-damage) have been reported in the colon and serum of IBD patients and fecal MPO is considered a marker of severity (12). When mice were depleted of neutrophil by dextran sodium sulphate (DSS) prior to induction of colitis; no inflammation in the colon was observed which supports the substantial involvement of neutrophil/MPO driven oxidants in pathogenesis of IBD. In Crohn’s disease and ulcerative colitis neutrophil infiltration is a striking feature particularly in cases of disease reversion (13). The ROS driven by pro-inflammatory cell infiltration into gastrointestinal mucosa impairs the redox balance essential to physiological cellular metabolism leading to oxidative stress (OS). Nitroxides possess strong antioxidant activity with low toxicity in humans and animals.

These are cell permeable which potentially inhibit the MPO/H2O2/Cl- system and thus inflammation (14, 15). For example, 4-hydroxy-2, 2, 6, 6-tetramethyl-piperidin-1-yl-oxyl (TEMPOL) inhibits indomathacin-induced gastric damage in rats (16). Nitroxides can maintain the cellular/tissue redox balance by scavenging the oxidants by several mechanisms. E. g. TEMPOL has shown a dose dependant in vivo and in vitro inhibition in oxidative damage (17). This exhibits peroxidase/catalase activity to degrade H2O2 and reduces accumulation of ROS. Nitroxides also inhibit damage induced by NO.-derived reactive species.

For example TEMPOL inhibits MPO-mediated RNase and protein nitration and reduces NO2. to nitrate (NO2-) in a cycle that regenerates active TEMPOL (18, 19). In pilot studies (at our group) administration of cyclic nitroxide 4-MetT (15 mg/kg bw) has shown improvement in body weight and clinical fecal scores in DSS induced colitis mouse model. Cyclic nitroxide 4-MetT also showed inhibition of MPO activity and decreased colon 3-nitrotyrosine levels (unpublished). Overall, it ameliorated colitis and attenuated pro-inflammatory stimuli and markers of protein and lipid oxidation. Nitroxides are substrates for MPO and are rapidly oxidized by MPO compound I via one-electron transfer to yield the pophyrin pi. cation radical and then MPO-compound II, thereby, inhibiting HOCL production and effectively peroxidase cycling. In this context we propose to investigate the role of nitroxide(s) as potent ani-inflammatory agents to ameliorate inflammatory bowel disease (IBD) in severe combined immune-deficient mouse model which recapitulate human IBD with following aims: AIM 1: Demonstrate that neutrophil/MPO

driven production of oxidants is central to pathogenesis of IBD in SCID mouse model of chronic colitis. AIM 2: Identify whether cyclic nitroxides (TEMPOL, 4-MetT) inhibit the neutrophil recruitment in inflamed colon and can substantially prevent the disease progression. AIM 3: Trace the precise temporal onset of disease using bioluminescence in vivo imaging technique(s) and to relate the course of disease with the possible protective role of supplemented nitroxide(s)AIM 4: Identify and select an optimal dose of best effective nitroxide to be used as a free-drug or delivered in nano-particles in chronic model of IBD which could be translated into clinical practice. Research PlanSelection of Animal Model for IBD InductionA major limitation in the understanding of pathophysiology and etiology of IBD has been the availability of acceptable animal model which recapitulate human disease. By far the most widely tested chemical model of IBD is dextran sodium sulphate (DSS) induced colitis and among gene-targeted models of spontaneous colitis is the IL-10 deficient (IL–10-) mouse.

An important caveat in DSS induced colitis is; unlike human IBD; T and B cells are not required to induce the disease which limits its recapitulation to human IBD (20). Likewise in IL-10 deficient IBD mouse model the onset and severity of disease are variable and disease may require months to develop which questions its feasibility as ideal experimental model. Further, in breeding of these mice (IL-10-/-) results in a significant reduction in the penetrance and severity of disease over period (21). In view of the above limitations and with the quest to acquire more precise synchronization of the onset and severity of the disease, the T cell transfer model of chronic colitis

has been widely studied over the last decade. This is considered the best characterized and pro-typical model of chronic colitis induced by disruption of T cell balance. Adoptive transfer of splenic CD4+CD45RBhigh T cells (naïve T cells) obtained from wild type healthy mice into severe combined immune-deficient (SCID) mouse lacking T and B cell induces pancolitis and small bowel inflammation 5-8 weeks post T cell transfer (22). The major advantage of this model is that it provides more accurate and well characterized time course of initiation of the disease. Further, this model is ideal to investigate the role of regulatory T cell in suppressing the onset of the disease (22, 23).

Wight loss and pathological outcomes of this model closely resemble the observations in human and most therapies that are effective in human IBD are also efficacious in this model. Induction of DiseaseDisease is induced by injecting purified splenic CD4+CD45RBhigh T cells harvested from wild type mice (C57BL/6) into SCID mice that lack T/B cells. The injected cell population is devoid of regulatory T cells (CD4+CD45RBlow) which are responsible to suppress the onset of disease. An imbalance of these cell (CD4+) populations leads to colonic inflammation mediated by IFN-γ and TNF production leading to immune responses that promote IBD (24). Chronic colitis does not develop in this mouse model under germ free conditions which indicate that CD4+CD45RBhigh cells generate an immune response against commensal bacteria if regulatory T cells are absent which is similar to human IBD.