INDOXYL SULFATE AFFECTS IMMUNE RESPONSE AND INTESTINAL HOMEOSTASIS IN MICE REGULATING INFLAMMATORY RESPONSE AND OXIDATIVE STRESS

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Introduction: Inflammatory and oxidative stress status play a pivotal role in Chronic Kidney Disease (CKD) associated complications. CKD is related to the progressive retention of a large number of compounds which, under normal conditions, are excreted by healthy kidneys. These compounds are called uremic toxins for their harmful effects in various physiological functions in CKD patients. Indoxyl Sulfate (IS) is a protein bound uremic toxin, poorly eliminated by dialysis. Intestine has a primary role in IS production: tryptophan is metabolized into indole by intestinal bacteria and after intestinal absorption, is further converted into IS in the liver. Evidences indicate the gastrointestinal tract as a major source of chronic inflammation in CKD and macrophage are involved in immune response and in oxidative stress. In this study we evaluated the effect of IS, at concentrations observed in CKD patients, on inflammatory and oxidative stress parameters both in primary macrophages and at intestinal level in mice. Moreover, among the various uremic toxins present in CKD serum patients, we studied the IS effect on intestinal epithelial cell line IEC-6.

Material and methods: c57BL/6J mice were treated with IS (800 mg/kg i.p.) or with vehicle. After 3or 6hours the animals were sacrificed and IS serum levels were evaluated. In primary peritoneal macrophages cycloxygenase-2(COX-2), inducible nitric oxide synthase (iNOS) and heme oxygenase-1(HO-1) expression as well as reactive oxygen species (ROS) release and nitrotyrosine formation, also in condition of Lipopolysaccharide from E.coli (LPS; 1μg/mL) stimulation, were evaluated by cytofluorimetric analysis. The histopathological analysis and the immunohistochemistry for COX-2and nitrotyrosine in mice intestinal tissue were also performed. To study the specific contribute of IS to oxidative stress we evaluated ROS release in intestinal epithelial cells (IEC-6) treated with sera, also treated with AST-120 - an IS adsorbent, derived from healthy people, CKD patients and CKD dialyzed patients.

Results: Our results indicate that IS serum levels significantly increased after 3h from its administration in mice. The pro-inflammatory and oxidative stress parameters (COX-2, iNOS, ROS, nitrotyrosine and HO-1) were significantly increased after 3and 6h from IS administration in mice primary macrophages. In inflammatory conditions these parameters resulted further increased in macrophage from IS-treated group mice after 6h. The histopathological analysis of the intestinal tissue indicated slight mucosal alterations and inflammation in IS- treated mice compared to the vehicle group. The immunohistochemistry analysis showed a greater number of COX-2and nitrotyrosine positive cells in the axis of the intestinal villi of IS-treated mice compared to vehicle group. In IEC-6cells ROS release was weakly influenced by human healthy people serum, but resulted increased in the cells treated with sera derived from CKD patients and mostly in cells treated with sera from CKD dialysed patients ($p<0.05$vs untreated cells). Moreover, AST-120 serum treatment significantly decreased ROS release from IEC-6cells ($p<0.01$vs IS alone).

Discussion and conclusions: Our results indicate that IS significantly contributes to the systemic inflammatory state and oxidative stress observed in CKD patients both affecting immune response, as shown in macrophage, and intestinal homeostasis, as shown in intestinal tissue and in IEC-6. These effects were further enhanced in macrophages during inflammatory conditions, often occurring in CKD patients. Moreover we found a correlation between IS serum concentration and ROS production in IEC-6cells, thus highlighting the significant pro-oxidant effect of IS, among the different uremic toxins accumulating in CKD, as shown by AST-120 cell treatment. Our results highlight that IS significantly contribute to CKD-associated alterations by regulating inflammatory and oxidative stress response.