Nephrol Dial Transplant (2006) 21: 2057–2060
doi:10.1093/ndt/gfl281
Advance Access publication 7 June 2006

Intestinal bacterial microflora—a potential source of chronic inflammation in patients with chronic kidney disease

Peter Kotanko, Mary Carter and Nathan W. Levin

Renal Research Institute, New York, NY, USA

Keywords: bacterial translocation; chronic inflammation; chronic kidney disease; gut; intestinal microflora

Introduction

Inflammation is prevalent in a large proportion of dialysis patients, irrespective of the dialysis modality used. *Central catheters*, *periodontal disease*, exposure to endotoxins from *non-ultrapure water* dialysis and persistent *chronic infections* are well-established causes of chronic inflammation, but many infections go unrecognized [1].

Despite the fact that 10–100 trillion microorganisms populate the intestine in adult humans, the *gastrointestinal tract* has received little attention as a possible source contributing to the chronic inflammation noted in dialysis patients.

The mucosal surface is the physical interface of the immune system with the outside world, encompassing an extensive surface area of 300–400 m^2, which constitutes the largest body interface between the host and microorganisms. The intestinal barrier is composed of different domains [2], the ecological barrier (the normal intestinal microflora), the mechanical barrier (single layer of polarized intestinal epithelial cells, the enterocytes, covered by mucus) and the immune barrier [secreted immunoglobulin A (IgA), immune cells including intraepithelial lymphocytes and macrophages, neutrophils, natural killer cells, Peyer's plaques and mesenteric lymph nodes]. The gut also harbours the largest and most diverse ecosystem of microbes in the human body, consisting of more than 400 species of bacteria. The bacterial diversity within the human colon is greater than previously thought [3]. Absolute bacterial counts vary along the length of the bowel, increasing from a concentration of $\sim 10^8$ organisms/ml intestinal fluid in the distal ileum to $\sim 10^{12}$ organisms/ml beyond the ileocecal valve. A rapid rate of enterocyte turnover helps to maintain the integrity of the intestinal barrier [4].

Commensal microorganisms play a crucial role in maintaining physiological bacterial–host interactions at the intestinal surface.

Bacterial translocation

'Bacterial translocation' describes the passage of viable resident bacteria and of macro-molecules such as lipopolysaccharide endotoxin across the intestinal barrier to the blood. There are two pathways for the passage of substances from the intestinal lumen to the blood, a paracellular and a transcellular route. Specific membrane pumps and channels govern the transcellular transport, whereas tight junctions control the paracellular pathway [5].

Numerous insults, such as infections, both in the intestinal tract and in other sites (such as pneumonia), inflammatory bowel disease, parenteral nutrition, malnutrition, surgical stress, burns, shock, obstructive jaundice, thermal injury, stress, circulatory compromise, congestion in heart failure and hypoxia, bacterial overgrowth and reduced intestinal motility may be causative of impaired intestinal barrier function [2]. Endotoxin concentrations were shown to be higher in oedematous than in stable congestive heart failure (CHF) patients. Gut-derived endotoxin may trigger immune activation and inflammatory responses in CHF patients during oedematous episodes [6].

Pathogens impair the integrity of the intestinal barrier with a number of different virulence factors. *Escherichia coli*, *Salmonella typhimurium*, *Clostridium perfringens*, *Bacteroides fragilis*, *Vibrio cholerae* and rotavirus directly disrupt tight-junction proteins [5]. Transcellular migration of *E. coli* and *Proteus mirabilis* have been visualized within intact enterocytes [7]. Host factors have an effect on bacterial proliferation and mucosal adhesion. Stress mediators such as norepinephrine and adrenocorticotropic hormone have been shown to directly enhance the virulence characteristics and adherence of enterohaemorrhagic *E. coli* to the colonic mucosa [8,9]. It is conceivable that sympathetic

Correspondence and offprint requests to: Nathan W. Levin, Renal Research Institute, 207 East 94th Street, Suite 303, New York, NY 10128, USA. Email: nlevin@rriny.com

overactivity, as observed in uraemia, may also alter intestinal susceptibility to bacterial translocation.

The cellular processes underlying bacterial translocation have been studied in the rat common bile duct ligation model. In this model xanthine oxidase (XO) is activated presumably by translocated bacterial products and XO-derived reactive oxygen species (ROS) are significantly increased. Inhibition or inactivation of XO by allopurinol or by a tungsten-supplemented diet normalized the mucosal ROS and attenuated bacterial translocation significantly [10]. ROS activate nuclear factor kappa B (NFkB) and the expression of genes encoding cytokines such as tumour necrosis factor-α (TNFα), interleukin-2 (IL2), IL8, intercellular adhesion molecule-1 (ICAM-1), and others. Some of these cytokines may promote a disruption of tight junctions and facilitate bacterial translocation [11].

Intestinal microflora, intestinal barrier function and bacterial translocation in uraemia

In uraemia, greatly increased concentrations of urea, creatinine and other nitrogenous metabolites reach the gut and become subject to microbial metabolism. Uraemic patients show greatly increased counts of both aerobic ($\sim 10^6$ bacteria/ml) and anaerobic ($\sim 10^7$ bacteria/ml) organisms in the duodenum and the jejunum, sites with very low or no intestinal bacterial counts in healthy subjects [12]. Intestinal bacteria are involved in the generation of uraemic toxins such as indoxyl sulphate and p-cresol, and the latter has recently been linked with mortality in dialysis patients [13].

There is evidence of impaired intestinal barrier function in uraemia. Magnusson *et al.* reported an increased intestinal permeability to differently sized polyethylene glycols (range 326–1254 Da) in uraemic rats [14] and chronic kidney disease (CKD) patients [15]. Constipation, a frequent problem in uraemic patients [16], promotes bacterial overgrowth, which in turn may increase intestinal barrier permeability and promote bacterial translocation. Bacterial translocation was recently reported in a rat 5/6 nephrectomy model [17]. In this study, bacterial translocation occurred at day 60 in 8/20 uraemic animals as compared with 1/20 controls ($P = 0.02$). Translocation in uraemic rats was observed in samples of the mesenteric lymph nodes (all eight cases) and of blood (two cases). No data are available on bacterial translocation in CKD patients. As mentioned earlier, the constant and rapid renewal of the epithelial lining of the digestive tract is essential to maintain the integrity of the intestinal barrier and the small intestinal epithelial lining is normally replaced every 3–6 days in humans [4]. Malnutrition impairs enterocyte turnover and may thus contribute to the breakdown of the intestinal barrier function.

Oral iron—a potential stimulus for intestinal bacterial growth

Iron is an important growth factor for bacteria (Table 1), and many bacteria produce siderophores to attract iron away from the host iron-binding proteins transferrin and lactoferrin [18]. Mammals lack a regulated means to excrete iron, and therefore dietary iron content and intestinal iron absorption defines iron stores. Individuals with iron overload, whether induced by excess dietary iron intake or due to diseases, such as β-thalassaemia major, haemochromatosis and sickle cell disease, are more susceptible to infection [19,20]. While oral iron supplementation has been shown clearly to be disadvantageous in certain settings such as in malarious regions [21], it is not clear whether oral iron supplementation increases the susceptibility of dialysis patients to infection. Interestingly, intestinal iron deposition is a frequent finding in uraemia, presumably because of reduced iron uptake [16]. Intravenous (i.v.) iron in haemodialysis patients is associated with an increased risk of infection [22]. Intravenous iron undergoes biliary secretion and may contribute to the intestinal iron load [23]. In chronically inflamed patients, intestinal iron uptake is blocked via the hepcidin pathway. The decrease in plasma iron levels during inflammation is a protective response of the body to combat the infection and to limit oxidative damage. As hepcidin is an acute-phase protein, it sequesters the body's iron stores and prevents this iron from being requisitioned by bacteria [24]. When oral iron is given to an inflamed subject, e.g. a dialysis patient, the amount of iron accessible to intestinal bacteria increases. It is tempting to speculate that increased iron availability stimulates the proliferation of intestinal bacteria and increases the production of gut-derived uraemic toxins and bacterial translocation. Moreover, unabsorbed iron may act as a catalyst in the production of hydroxyl radicals [25]. Reducing intestinal iron availability

Table 1. Bacteria that require iron for growth and whose virulence is enhanced by excess iron (adapted from [20])

Gram-negative bacteria	Gram-positive bacteria
Acinetobacter	Bacillus
Aeromonas	Clostridium
Campylobacter	Corynebacterium
Capnocytophaga	Erysipelothrix
Chlamydia	Listeria
Ehrlichia	Staphylococcus
Escherichia	Streptococcus
Klebsiella	
Legionella	
Moraxella	
Neisseria	
Pasteurella	
Proteus	
Pseudomonas	
Salmonella	
Shigella	
Vibrio	
Yersinia	

in inflamed patients may therefore be beneficial. Iron-binding tannins in tea, phytic acid in bran and synthetic oral iron chelators such as deferiprone reduce the intestinal free-iron concentration.

Strategies to decrease intestinal permeability and bacterial translocation

Nutrients protecting the gastrointestinal tract and maintaining the integrity of the intestinal barrier have received great attention in recent years [26]. Glutamine, arginine, zinc, vitamin A, probiotics (live microorganisms in fermented foods that establish and improve the intestinal microflora) and prebiotics (non-digestible food ingredients, that beneficially affect the host by selectively stimulating the growth and activity of a limited number of bacteria in the colon) have been tested in multiple trials. Although many of these trials have been criticized on methodological grounds [26], current evidence points towards a beneficial effect of these interventions on intestinal barrier function. In a prospective trial on patients with CHF, intensified diuretic treatment reduced intestinal congestion and normalized endotoxin concentrations [6]. No interventional trial on gut permeability is available in humans with impaired renal function. In 5/6 nephrectomized rats, probiotics treatment improved azotaemia [27]. Studies are limited by the tools available to follow changes of intestinal microflora dynamically. New technologies, such as analysis of volatile organic compounds by selected ion flow tube mass spectrometry (SIFT-MS) [28] and molecular biology [3] approaches may prove to be particularly useful in studying the intestinal microbiology in uraemic patients.

Conclusion

Intestinal bacteria contribute to the uraemic syndrome by the production of uraemic toxins. Additional evidence suggests that translocation of bacteria and endotoxins from the gut to the blood takes place in kidney failure. Consequently, it is plausible to assume that the gut contributes to the chronic inflammatory state in dialysis patients. The availability of iron in the intestinal lumen may increase growth and virulence of intestinal bacteria and affect the intestinal barrier adversely. Oral iron chelation may be beneficial in reducing the intestinal iron load. Basic research and clinical studies are needed to further define the significance of intestinal bacteria and their products in uraemia. Interventions aimed at restoring and maintaining the physiological intestinal microflora in dialysis patients should be tested rigorously in clinical trials.

Conflict of interest statement. None declared.

References

1. Yao Q, Axelsson J, Heimburger O *et al*. Systemic inflammation in dialysis patients with end-stage renal disease: causes and consequences. *Minerva Urol Nefrol* 2004; 56: 237–248
2. Ding LA, Li JS. Gut in diseases: physiological elements and their clinical significance. *World J Gastroenterol* 2003; 9: 2385–2389
3. Eckburg PB, Bik EM, Bernstein CN *et al*. Diversity of the human intestinal microbial flora. *Science* 2005; 308: 1635–1638
4. Williamson RC. Intestinal adaptation (first of two parts). Structural, functional and cytokinetic changes. *N Engl J Med* 1978; 298: 1393–1402
5. Baumgart DC, Dignass AU. Intestinal barrier function. *Curr Opin Clin Nutr Metab Care* 2002; 5: 685–694
6. Niebauer J, Volk HD, Kemp M *et al*. Endotoxin and immune activation in chronic heart failure: a prospective cohort study. *Lancet* 1999; 353: 1838–1842
7. MacFie J. Current status of bacterial translocation as a cause of surgical sepsis. *Br Med Bull* 2004; 71: 1–11
8. Green BT, Lyte M, Chen C *et al*. Adrenergic modulation of *Escherichia coli* O157:H7 adherence to the colonic mucosa. *Am J Physiol Gastrointest Liver Physiol* 2004; 287: G1238–1246
9. Schreiber KL, Brown DR. Adrenocorticotrophic hormone modulates *Escherichia coli* O157:H7 adherence to porcine colonic mucosa. *Stress* 2005; 8: 185–190
10. Schimpl G, Pabst MA, Feierl G *et al*. A tungsten supplemented diet attenuates bacterial translocation in chronic portal hypertensive and cholestatic rats: role of xanthine dehydrogenase and xanthine oxidase. *Gut* 1999; 45: 904–910
11. Weber-Mzell D, Zaupa P, Petnehazy T *et al*. The role of nuclear factor-kappa B in bacterial translocation in cholestatic rats. *Pediatr Surg Int* 2006; 22: 43–49
12. Simenhoff ML, Saukkonen JJ, Burke JF *et al*. Bacterial populations of the small intestine in uremia. *Nephron* 1978; 22: 63–68
13. Bammens B, Evenepoel P, Keuleers H *et al*. Free serum concentrations of the protein-bound retention solute p-cresol predict mortality in hemodialysis patients. *Kidney Int* 2006; 69: 1081–1087
14. Magnusson M, Magnusson KE, Sundqvist T *et al*. Increased intestinal permeability to differently sized polyethylene glycols in uremic rats: effects of low- and high-protein diets. *Nephron* 1990; 56: 306–311
15. Magnusson M, Magnusson KE, Sundqvist T *et al*. Impaired intestinal barrier function measured by differently sized polyethylene glycols in patients with chronic renal failure. *Gut* 1991; 32: 754–759
16. Kang JY. The gastrointestinal tract in uremia. *Dig Dis Sci* 1993; 38: 257–268
17. de Almeida Duarte JB, de Aguilar-Nascimento JE, Nascimento M *et al*. Bacterial translocation in experimental uremia. *Urol Res* 2004; 32: 266–270
18. Wandersman C, Delepelaire P. Bacterial iron sources: from siderophores to hemophores. *Annu Rev Microbiol* 2004; 58: 611–647
19. Walter T, Olivares M, Pizarro F *et al*. Iron, anemia, and infection. *Nutr Rev* 1997; 55: 111–124
20. Ashrafian H. Hepcidin: the missing link between hemochromatosis and infections. *Infect Immun* 2003; 71: 6693–6700
21. Oppenheimer SJ. Iron and its relation to immunity and infectious disease. *J Nutr* 2001; 131: 616S–633S
22. Canziani ME, Yumiya ST, Rangel EB *et al*. Risk of bacterial infection in patients under intravenous iron therapy: dose versus length of treatment. *Artif Organs* 2001; 25: 866–869

23. Brissot P, Bolder U, Schteingart CD *et al*. Intestinal absorption and enterohepatic cycling of biliary iron originating from plasma non-transferrin-bound iron in rats. *Hepatology* 1997; 25: 1457–1461
24. Robson KJ. Hepcidin and its role in iron absorption. *Gut* 2004; 53: 617–619
25. Lund EK, Wharf SG, Fairweather-Tait SJ *et al*. Oral ferrous sulfate supplements increase the free radical-generating capacity of feces from healthy volunteers. *Am J Clin Nutr* 1999; 69: 250–255
26. Duggan C, Gannon J, Walker WA. Protective nutrients and functional foods for the gastrointestinal tract. *Am J Clin Nutr* 2002; 75: 789–808
27. Ranganathan N, Patel BG, Ranganathan P *et al*. *In vitro* and *in vivo* assessment of intraintestinal bacteriotherapy in chronic kidney disease. *Asaio J* 2006; 52: 70–79
28. Smith D, Spanel P. Selected ion flow tube mass spectrometry (SIFT-MS) for on-line trace gas analysis. *Mass Spectrom Rev* 2005; 24: 661–700

Received for publication: 25.4.06
Accepted in revised form: 25.4.06

Nephrol Dial Transplant (2006) 21: 2060–2063
doi:10.1093/ndt/gfl219
Advance Access publication 23 May 2006

Nephrotoxicity of ciclosporin A: short-term gain, long-term pain?

Jeremy R. Chapman and Brian J. Nankivell

Centre for Transplant and Renal Research, Millenium Institute, Westmead Hospital, University of Sydney, Australia

Keywords: chronic allograft nephropathy; ciclosporin; nephrotoxicity

Historical perspective

Ciclosporin (CsA) was first dosed in pilot renal transplant recipients at 25 mg/kg/day, based upon large animal experimental data [1], but was rapidly found to be nephrotoxic and the dose reduced to 10 mg/kg/day. Monotherapy at this dose was, however, found to provide inadequate immunosuppression [2] and a starting dose of 17.5 mg/kg/day was thus used in the early phase II/III of clinical studies [3,4]. This early experience changed the clinical practice of transplantation, since acute rejection became a more subtle clinical syndrome and a differential diagnosis of acute nephrotoxicity had to be considered for renal dysfunction. The therapeutic window of CsA, when used alone, proved too narrow and thus, triple therapy, including azathioprine and low-dose prednisolone was born in the late 1980s [5]. The histological picture of acute CsA nephrotoxicity was defined both in transplanted and native kidneys, with the widely agreed hallmarks being striped or diffuse interstitial fibrosis, nodular arteriolar hyalinosis and tubular calcification [6].

Correspondence and offprint requests to: Jeremy R. Chapman, Centre for Transplant and Renal Research, Millenium Institute, University of Sydney, Westmead Hospital, Westmead NSW 2145, Australia. Email: jeremy_chapman@wsahs.nsw.gov.au

Acute nephrotoxicity

The functional impact of CsA nephrotoxicity appeared in the early formal randomized clinical trials [3,4,7] with impaired tubular function, higher serum urate levels and altered potassium handling. Blood pressure was increased and glomerular filtration rate (GFR) was reduced, but rapid recovery to control levels was observed after cessation of the drug. Synergisitic toxicity with non-steroidal anti-inflammatory agents hinted at the vascular nature of the functional insult caused by CsA. Histological confirmation of CsA nephrotoxicity was seldom forthcoming in patients with acute rises in serum creatinine and clinicians learnt to rely for diagnosis, on an absence of the histological features of rejection together with a response to dose reduction. The reassurance that CsA nephrotoxicity was reversible—at least after 3 months of treatment—was encouraging, but the words of warning in those early papers with respect to uncertainty over reversibility in the longer term, went unheeded [7]. Short-term graft survival rates continued to improve throughout the 1990s and little attention was paid to the longer-term outcomes.

Chronic nephrotoxicity

The concern that chronic calcineurin inhibitor (CNI) nephrotoxicity was a major long-term problem should have been widely understood in the mid-1990s through