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Are *Clostridium difficile* toxins nephrotoxic?

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ABSTRACT

Clostridium difficile-associated disease (CDAD) occurs along a spectrum from simple uncomplicated enteritis to a multi-system disease which may include nephropathy. Pathology is attributed to bacterial toxins, but it is unclear if the latter are directly nephrotoxic. Anecdotes of renal disease from human biopsy findings suggest a variation of histopathologies, but data are relatively limited. Acute renal failure does occur in patients with advanced morbidity. CDAD can complicate chronic renal failure. Kidney tissue culture cytotoxicity has long been known. Kidney function alterations among animal models or diseased humans are relatively uncommon in mild to moderate enteritis. Rare findings of toxinemia are reported. Some have proposed that renal dysfunction arises more from pre-renal compromises. Direct toxin studies on whole kidney are sparse. The role of direct toxin-associated renal disease is worthy of further investigation given the current impetus towards the development of protective and therapeutic passive and active immunity. Hypotheses of toxin-direct or pre-renal toxin compromise of renal function prevail.

Introduction

Clostridium difficile is a common human enteric pathogen. Disease may vary from mild diarrheal symptoms to fulminant pancolitis, and many associated systemic manifestations including death have been detailed. Contemporary concerns about the frequency of infection, epidemic spread, treatment, and prevention continue to be sources for medical concern, and there is evidently a plethora of research which continues on these themes worldwide. Many large clinical reviews of *C. difficile*-associated disease (CDAD) have emerged since the cause-and-effect relationship between bacterium/toxins and disease was established in the late 1970s [1–5].

Central to the understanding of pathogenesis was the recognition of the important exotoxins, TcdA and TcdB [6–8]. Tcd A was generally considered to be an enterotoxin by historic definitions, whereas TcdB had been defined as a classical cytotoxin. These toxins work in concert, however, to effect cytoskeletal dysfunction. Both toxins bind to colonic epithelial cells and are thereafter internalized. They are then activated intracellularly and biochemically modulate Rho cellular proteins which regulate the functions of actin. Initial cell structure damage is followed by apoptosis. Although with some caveats, the majority of science points to a conjoined dysfunction of bowel (especially colonic) epithelial integrity and an upregulation of localized immunological reactions due to these combined toxins. The toxin theory of localized disease is forefront in disease causation, and the serious intestinal and non-intestinal sequelae have largely been ascribed to secondary physiological and pathological responses.

Kidney function in the context of *C. difficile*-associated disease had assumed interest in several regards as further outlined herein, and indeed the potential for acute renal failure in the context of serious CDAD is well-described. Despite the latter, kidney dysfunction in the presence of mild to moderate disease seems to have been uncommonly detailed. Many large studies and reviews of CDAD do not indicate that it is commonly associated with either acute or chronic nephropathy [1–5,9]. The following case study illustrates the nature of concern for the nephrotoxicity of *C. difficile* toxins in relevance to the central hypotheses.

A 68 yr. male was attended to for mild diarrheal symptoms which then raised concern for CDAD. He had undergone placement of a mechanical aortic valve and pacemaker some years prior. He developed prosthetic valve endocarditis with methicillin-susceptible *Staphylococcus aureus* and endured a complicated hospital course which included pleural effusions, congestive heart failure, hemothorax, enterococcal empyema, anemia, and acute renal failure which required temporary hemodialysis. Past medical co-morbidities had included dyslipidemia, ischemic heart disease, and medical anticoagulation, but renal function had been normal prior to the endocarditis. In hospital, he

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at times had received piperacillin/tazobactam, vancomycin, cloxacillin, rifampin, and linezolid. After hospital discharge, he was prescribed oral rifampin and levofloxacin for a six-month period to complete the extended treatment for the endocarditis given the situational inability to undergo surgical valve replacement. Clinically and microbiologically, he was cured. Four weeks after completion of the oral antibiotic regimen, he suffered diaphoresis and an apparent mild gastrointestinal illness; there was no fever. Stool analyses determined the presence of *C. difficile* toxin in the absence of any other enteric pathogen. Fecal leucocytes were present. There was no evidence of relapse with endocarditis. Prior to therapy of his enteric illness, the serum creatinine was elevated at 287 μmol/l. After ten days of oral metronidazole (500 mg bid), his bowel function normalized, and the creatinine decreased to 238 μmol/l. He relapsed with fever and mild enteritis after only a few days of completing the antibiotic regimen, and the creatinine had risen to 295 μmol/l. CDAD was again laboratory-confirmed. Within a few days of oral vancomycin, his clinical disease settled, and the creatinine reduced to 253 μmol/l. Throughout these illnesses, he appeared reasonably hydrated, and the colonic output was only modest at best.

In the setting of mild, severe, or relapsing disease, are *C. difficile* toxins directly nephrotoxic? Several hypotheses could account for pre-renal and/or direct renal toxicity, but is there clearly a cause-and-effect relationship?

Associations of *C. difficile* with renal disease

If *C. difficile* toxins were indeed nephrotoxic, one might envisage a dose-response effect analogous to that seen with verotoxigenic *Escherichia coli* [10]. For the latter, a higher incidence of severe disease, including nephropathy (hemolytic uremic syndrome [HUS]), was observed for younger children. Two large studies of pediatric populations with CDAD, however, do not support this view. In the series of Pai et al., only 2.9% of patients were found to have a rising creatinine during infection [11]. Schwartz et al. noted that 13% of children had laboratory-based renal compromise at the time CDAD was diagnosed [12]. The latter authors hypothesized that renal disease was more likely a function of other underlying co-morbidities rather than due to the CDAD itself. A third study among children found elevated age-adjusted serum creatinine levels for those who presented with active CDAD [13]. Multivariate analyses determined that older age was an independent risk factor for severe illness. The latter would run contrary to the pattern expected for the toxin hypothesis of CDAD-associated renal compromise since older age would generally carry a lower toxin-body weight ratio.

Despite the above, convincing citations of acute renal failure (ARF) that accompany CDAD exist [14–17]. ARF during illness is a marker for disease severity [18]. It also is a risk factor for adverse outcome [18,19]. ARF is associated with increased mortality in CDAD [16,20,21]. One retrospective review, however, did not find an association of creatinine levels and mortality [9]. Among the latter is an increased risk of death after therapeutic colectomy when ARF occurs [22,23]. Infection has been associated with increased deterioration of the transplanted kidney, and pre-existing chronic renal failure (CRF) predisposes the patient to ARF in the course of the illness [24,25]. Again, some authors hypothesize that the acute renal impact is more a function of pre-renal factors, especially hypovolemia due to the loss of fluids, serum proteins such as albumin, and electrolytes at the intestinal site [15,17].

CRF poses a risk for *C. difficile* colonization of the bowel [26]. It is also a risk factor for the occurrence of symptomatic CDAD and disease recurrences [27–29]. In patients with renal compromise, CDAD is associated with increased morbidity, increased mortality, greater length of hospital stay, and more hospital costs [29–31]. The incidence of CDAD among those with CRF does not appear to correlate with the degree of kidney dysfunction [32]. Treatment of CDAD has a greater risk for clinical failure when patients have pre-existing CRF [25].

Two studies have examined risk factors for CDAD among renal transplant patients [33,34]. In either study, there were no identifiable risk factors that would be seen as consistent with a toxin-induced secondary renal disease except that CDAD proved to be an independent risk factor for the consequence of an acute renal rejection episode [34].

Historic *in vitro* data

The discovery of *C. difficile* toxins was initially dependent on the demonstration of tissue culture cytotoxicity after exposure to stool and bacterial filtrates. Almost any cell line may be affected *in vitro* depending on the toxin titre that is applied. Kidney cell lines, however, have been specifically tested for susceptibility.

A cytopathic effect for toxins had been shown in tissue cultures of Vero (African green monkey kidney) and MA-104 (rhesus monkey kidney) cell lines [35,36]. Toxin A causes apoptosis in Vero cells and Madin-Darby canine kidney cells [37,38]. Toxin B is cytotoxic to human embryonic kidney (HEK) cells [39]. Such cell lines and others have served as the toxin substrate for many molecular discoveries in this field. These findings were evidently very crude indicators for direct nephrotoxic potential.

Unique renal pathology

A variety of nephropathological presentations have been cited on the basis of clinical and/or histological findings Table 1. Most of these are detailed as non-atypical HUS and among the latter, the majority were female. Some of the illnesses have mimicked HUS that is caused by verotoxigenic *E. coli* for which there has also been some debate of the association of female gender with the increased risk for progression [10,54,55]. One report describes the simultaneous detection of verotoxin and *C. difficile* toxin [56]. Others detail the occurrence of CDAD after treatment for verotoxigenic *E. coli* infection [57]. The citation of thrombotic microangiopathy must be tempered given the concomitant findings of systemic histoplasmosis [46]. Both oxalate nephropathy and renal hemosiderosis could be construed as complications of CDAD, albeit rare [50,52]. IgA nephropathy has been found in the context of CDAD, but also with enteric disease that is secondary to many other pathogens [43,58]. Acute tubular necrosis has been mentioned several times in these contexts [40,43,46,50–52]. There is no consensus that any one of the above nephropathies is representative of pathology during acute CDAD. Whereas the majority of these citations document the occurrence of CDAD-associated HUS, other diarrheal pathogens beyond *C. difficile* and VTEC have been associated with HUS [59]. The latter raises the hypothesis for common local bowel and complicating mechanisms rather than a unique bacterial specific toxin-directed disease. More consistent reporting from prospective biopsies and/or patient deaths is clearly needed to elucidate these issues.

Post-mortem animal and human findings

Post-mortem tissue has been available from both experimental animal models and human deaths. Infant monkeys were exposed to *C. difficile* toxin either through the intravenous or intraperitoneal routes, and they suffered demise [60]. Both toxin A and B exposures were associated with increases in serum creatinine up until the time of animal death, but the peak levels were relatively low even though a 100–200% change in values was determinable. Electrolyte alterations were much more dramatic. On pathology examinations, the appearance of kidneys was said to be unremarkable. In another study, both gnotobiotic piglets and mice received oral toxin [61]. Findings of renal pathology were amiss. Several CDAD-associated human deaths were subject to autopsy review, and kidney disease was not highlighted [62]. In a case report of pseudomembranous colitis and death, pathology was not identified among the internal organs outside of the bowel [63]. Given the

Table 1
Spectrum of renal disease associated with active CDAD.

Age	Gender	Prodromal	Renal				Year Ref.
		Illness	Disease	Treatment	Recovery	Other Unique Features	
20 mo.	F	bloody diarrhea	HUS	vancomycin	yes	toxin assay not performed, received antimotility agents	1988 [40]
11 yrs.	F	bloody diarrhea	HUS	chloramphenicol, supportive therapy	death	renal tissue confirmed	1988 [40]
51 yrs.	F	diarrhea	HUS	vancomycin	yes	plasma infusions	1997 [41]
32 yrs.	F	bloody diarrhea	HUS	metronidazole, colectomy	yes	recurrent disease, renal transplant kidney	1998 [42]
25 yrs.	F	diarrhea, hematuria	IgA nephropathy	metronidazole, vancomycin	yes	recurrent disease, also received methylprednisolone and cyclophosphamide	1999 [43]
46 yrs.	F	bloody diarrhea	HUS	metronidazole	yes	plasmapheresis and hemodialysis, renal biopsy confirmed	2003 [44]
11 yrs.	M	bloody diarrhea	HUS	supportive therapy	yes	renal biopsy confirmed	2004 [45]
50 yrs.	F	diarrhea	thrombotic microangiopathy	metronidazole	yes	renal transplant kidney, hemodialysis, also had acute histoplasmosis, renal biopsy confirmed	2005 [46]
preterm newborn	M	diarrhea, hematuria	HUS	metronidazole, probiotic	yes		2010 [47]
62 yrs.	F	emesis, abdominal pain	HUS	vancomycin	yes	dialysis, plasmapheresis	2012 [48]
73 yrs.	F	diarrhea	atypical HUS	metronidazole	yes	hemodialysis, IV steroid, called atypical HUS but had prodromal diarrhea	2012 [49]
69 yrs.	M	diarrhea	oxalate nephropathy	metronidazole, probiotic	yes	myelodysplastic syndrome, renal biopsy confirmed	2014 [50]
29 yrs.	F	diarrhea	HUS	metronidazole, vancomycin	yes	renal transplant kidney, renal biopsy confirmed	2014 [51]
52 yrs.	F	diarrhea	HUS	metronidazole	yes	renal transplant kidney, renal biopsy confirmed, required nephrectomy	2014 [51]
63 yrs.	F	bloody diarrhea	HUS	vancomycin, metronidazole	yes	hemodialysis and plasmapheresis, renal biopsy confirmed	2014 [51]
54 yrs.	M	bloody diarrhea	renal hemosiderosis	?	yes	HIV infected, hemodialysis, renal biopsy confirmed	2015 [52]
6 yrs.	M	diarrhea	atypical HUS	metronidazole, vancomycin	yes	hemodialysis, plasma exchange, eculimuzab infusions	2018 [53]

voluminous clinical CDAD that is mostly not reported scientifically from either community or hospital, it is inevitable that many deaths would have been recorded, and yet the details of reported pathology data are considerably sparse.

Correlates of toxinemia

The finding of bacterial toxin(s) in the bloodstream might at first glance give some credibility to a toxin hypothesis for renal disease. In animal models, toxin has been detected systemically, and the findings of such toxin correlate with worse experimental disease [61,64]. Circulating toxin could not be found in mild CDAD, and antitoxin appeared to be protective in some experiments. Foals with CDAD had some elevations in serum creatinine [65]. Human cytotoxinemia reports are rare, but it is unclear how much this has been assessed in infected populations generally [62].

Anti-motility pharmacological agents given orally to impede the diarrheal state can be a risk for worsening CDAD [66,67]. In the science of verotoxigenic *E. coli*, anti-motility drugs are a risk factor for some complications of initial infection, and it is speculated that this effect is prompted by increasing toxin exposure and absorption due to bowel stasis [10,54]. Emergency colectomy can lessen CDAD mortality, implying that removal of a toxin source can lessen systemic exposure [68]. In another correlate of the latter, bowel lavage apparently prevented the need for colectomy [69]. Although the latter could be consistent with a toxin-associated illness, the benefits of bowel lavage or colectomy could also be attributed to the diminution of local inflammation, secondary bacterial sepsis, or endotoxinemia. Colectomy may also be effective when the advanced nature of intestinal epithelial damage is irreversible. The lack of finding circulating toxin does not negate the nephrotoxin hypothesis, since it is not clear if indeed toxin unmeasurable by cytotoxicity assays can be transported to active disease sites systemically. Neither is it evident whether continuous exposure to toxin in subcytotoxic levels can amount any tissue pathology.

In another perspective, reports have emerged of high circulating levels of vancomycin in some patients with pre-existing renal disease when they are treated with oral vancomycin [70,71]. Given vancomycin's potential for renal compromise, the possibility for iatrogenic nephrotoxicity must be considered as a complicating feature of treatment.

Toxin immunity and antitoxin therapy

An immune response to *C. difficile* toxin occurs after infection [72]. Low toxin A antibody titres at the time of CDAD diagnosis are associated with greater morbidity [20]. Toxin A antibody naturally increases after infection, and this humoral response is protective [73,74]. The potential value of passive and active humoral immunity has garnered much interest [75–77]. Passive immunotherapy with animal or human donors has been assessed in part [78,79]. Oral bovine immunoglobulin treatments have garnered attention as a treatment for active CDAD [80,81]. A logical progression thereafter would be to develop antitoxin vaccines or to construct neutralizing monoclonal antibodies for clinical use [82–85]. It may be questioned rightfully, however, as to whether protective circulating antitoxin tactics, in which ever way devised, have a beneficial role by neutralization mainly at the bowel level, systemically, or both. None of the aforementioned studies clarifies the issue of nephrotoxicity directly, nor is there any science to refute the potential for neutralized toxin complexes to cause renal disease by any other mechanism.

Direct toxin studies

In a mouse model of oral toxin exposure, death can be achieved depending on the administered dose [86]. Toxin A can induce leucocyte-dependent leakage of albumin from bowel circulation via microvascular effects [87]. In a Golden Syrian hamster model of spontaneous

C. difficile infection, animals with acute diarrhea had grossly normal kidneys [88]. A subset of the latter animals developed chronic diarrheal illnesses. For the latter, the kidneys were macroscopically abnormal, and large deposits of amyloid were found in renal glomeruli and basement membranes. In other studies of hamster CDAD, amyloid could not be found [89]. These competing findings suggested that amyloid may have been but a remnant of pathology from chronic disease.

Monteiro et al. examined the effect of direct toxin A infusion on the isolated rat kidney [90]. Toxin A decreased renal perfusion pressure, decreased glomerular filtration rate, had no effect on tubular sodium transport, and increased urinary outflow in a time-dependent manner. Details of histopathological change were not assessed. No other studies of direct toxin effects on kidneys have been reported.

Conclusion

Despite the data detailed above, there is generally a lack of strong evidence to deny a toxin hypothesis of direct renal pathology in CDAD. Many clinicians and scientists appear content with the notion that acute renal failure in this context is initiated predominantly by pre-renal factors at the time when bowel disease is advanced. Another tenable hypothesis is that both routes of pathology may factor into renal compromise depending on clinical variables. Research in this area of CDAD would be timely given the thrust to develop passive and/or active antitoxin immunotherapy.

Compliance with ethical standards:

- there are no conflicts of interest in any regard
- the review does not invoke human or animal research de novo
- informed consents are not required for this review

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2019.03.002.

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