Findings that shed new light on the possible pathogenesis of a disease or an adverse effect

Microbes without frontiers: severe haemolytic-uraemic syndrome due to *E coli* O104:H4

Sophie Binks,¹ Kate Regan,² Jonathan Richenberg,^{1,3} Tim Chevassut⁴

¹Brighton and Sussex Medical School, Brighton, UK

²Intensive Care Unit, Royal Sussex County Hospital, Brighton and Sussex University Hospitals NHS Trust, Brighton, UK

³Department of Imaging and Nuclear Medicine, Royal Sussex County Hospital, Brighton and Sussex University Hospitals NHS Trust, Brighton, UK

⁴Department of Haematology, Royal Sussex County Hospital, Brighton and Sussex University Hospitals NHS Trust, Brighton, UK

Correspondence to Dr Tim Chevassut, T.Chevassut@bsms.ac.uk

Summary

Antibiotic use in infection with Shiga-toxin-producing strains of *Escherichia coli* (*E coli*) is thought to increase the risk of developing haemolytic-uraemic syndrome (HUS). One paediatric study concluded that *E coli* O157:H7-infected patients who had received antibiotic therapy were 17 times more likely to progress to HUS than those who had not. Quinolones are among those incriminated. In vitro experiments suggest toxin induction of 80-fold with ciprofloxacin and *E coli* O104:H4. We report here the case of a 44-year-old man returning from Hamburg who presented with a 5 day history of bloody diarrhoea which had worsened after starting ciprofloxacin. A severe illness of overlapping HUS and thrombotic thrombocytopenic purpura (TTP) ensued, with neurological complications requiring ventilation and intensive care admission. Stool sample eventually confirmed *E coli* O104:H4. Although the patient made a good recovery following treatment with haemofiltration and plasma exchange with fresh frozen plasma (FFP), ciprofloxacin may have exacerbated his clinical course.

BACKGROUND

Haemolytic-uraemic syndrome (HUS) is a rare but serious complication of *Escherichia coli* enteritis, with an outcome of mortality or end-stage renal failure in 12% of paediatric patients, a proportion which may be even greater in adults.^{1,2} In recent years, total annual UK infections with the most common Shiga-toxin-producing *E coli* strain of O157:H7 has ranged from 793 to 1182.³ At the onset, it may resemble other more benign gastrointestinal infections, for which ciprofloxacin is frequently prescribed.⁴

However, ciprofloxacin has demonstrated potential to aggravate infection with Shiga-toxin-producing *E coli* both in vitro and in vivo, particularly at subinhibitory levels. An 80-fold rise in Shiga-toxin-encoding genes was detected in O104:H4 colonies grown on ciprofloxacin-impregnated medium,⁵ while administration of this antibiotic to mice colonised with *E coli* O157:H7 triggered a rise in faecal toxin load and 66% mortality.⁴ Moreover, in a Danish case series of five O104:H4-HUS patients, four had previously received ciprofloxacin or metronidazole.⁶ The case described here also deteriorated after the start of ciprofloxacin. In addition, the ability of this class of drugs to lower the seizure threshold is of potential relevance to subsequent development of generalised tonic-clonic seizure. We present blood film findings in *E coli* O104:H4-associated HUS and unusual renal imaging features consistent with a toxin insult of gastrointestinal origin, which may help alert clinicians to this syndrome. Finally, the patient's improvement with plasma exchange and FFP may add to evidence for this treatment modality in HUS as well as its more established role in TTP.

CASE PRESENTATION

A 44-year-old man presented to the hospital with a 5-day history of bloody diarrhoea. He had recently travelled to Germany. Symptoms had worsened since he started taking ciprofloxacin 3 days back. On examination, he was afebrile and his abdomen was soft and non-tender with sparse bowel sounds. Despite adequate fluid resuscitation, the patient became anuric. Ciprofloxacin was discontinued due to possible differentials including *E coli*-mediated HUS. The day after admission, the patient developed confusion and a fluctuating conscious level culminating in a generalised tonic-clonic seizure necessitating intubation and admission to intensive care.

INVESTIGATIONS

Laboratory results on admission indicated acute kidney injury with creatinine of 308 µmol/l. There was neutrophilia of 31.1×10^9 cells/l and thrombocytopenia of 66×10^9 /l with normal clotting (INR 1.2 and APTT 1.1) and elevated fibrinogen (7.0 g/l). The next day, haemoglobin fell to 11.4 g/dl and platelet count to 52×10^9 /l. Lactate dehydrogenase was 2022 iu/l and blood film prior to start of plasma exchange demonstrated giant platelets, toxic neutrophils and fragmented red blood cells (figure 1). Contrast CT revealed bowel wall thickening (figure 2A), gross ascites and hypoperfused renal cortices (figure 2B).

DIFFERENTIAL DIAGNOSIS

This case presented during the 2011 German outbreak of *E coli* O104:H4, creating a high index of suspicion for *E coli*-mediated disease. By serendipity, we were made aware

Figure 1 The patient's blood film demonstrated giant platelets, toxic neutrophils and fragmented red blood cells.

of the outbreak of *E coli* in Germany prior to notification by the UK Health Protection Agency since the junior doctor involved with the case was told of a high number of HUS cases by her mother who worked on a dialysis unit in Hamburg. The main differential diagnosis considered at time of presentation was an acute initial flare of inflammatory bowel disease with possible sepsis, although this did not fit with subsequent neurological manifestations. Disseminated intravascular coagulation was considered unlikely due to normal clotting profile and raised serum fibrinogen.

An afebrile presentation with elevated white cell count has been reported as characteristic of *E coli*-associated HUS.⁷ Microangiopathic haemolytic anaemia, as evidenced by this patient's haemoglobin and LDH levels and blood film, is a feature of both HUS and TTP, with renal impairment more prominent in HUS and neurological complications more typical of TTP.² Despite this, there is considerable overlap in clinical presentation of these syndromes.⁸ Although low levels of the von Willebrand factor-cleaving protease ADAMTS-13 have recently been proposed to distinguish TTP from HUS,^{6 8} this assay is not yet in widespread use and rarely, if ever, alters immediate clinical management. Therefore, based on the presenting history and investigations a diagnosis of overlapping HUS-TTP was made.

TREATMENT

The patient was treated with haemofiltration and 8 days of plasma exchange with FFP and Octaplas (Octapharma, AG, Lachen, Switzerland).

OUTCOME AND FOLLOW-UP

The patient was intubated for 9 days and transferred from the intensive therapy unit (ITU) to a renal unit after successful extubation and neurological normalisation. He was discharged from hospital after more than 2 weeks with improving renal function attaining eGFR of 46 ml/min 2 weeks postdischarge.

DISCUSSION

The causative agent of *E coli*-associated HUS, Shiga-toxin 2, is encoded by a bacteriophage with both a lysogenic and a lytic phase.^{4 9} During the lytic phase, an increase in phage replication and bacterial host lysis leads to enhanced production and release of Shiga-toxin.^{4 9} One trigger of phage entry to the lytic phase is initiation of the bacterial 'SOS' response, which may be triggered by antibiotics targeting DNA synthesis, such as quinolones.^{4 10} Similar problems are encountered with other phage-encoded toxins, such as staphylococcal Pantone-Valentine leucocidin (PVL), which through an effect on certain regulatory regions is transcriptionally upregulated by β -lactam antibiotics.^{11 12}

In vitro experiments have confirmed up to 200-fold Shiga-toxin increase with the quinolone drugs ofloxacin, nalidixic acid and ciprofloxacin and *E coli* O157.¹⁰ Moreover, phage induction and high levels of Shiga-toxin were detected in faecal samples of *E coli* O157:H7-infected mice given ciprofloxacin, but not fosfomycin-treated or control animals.⁴ The O104:H4 and O157:H7 phages have highly similar genetic profiles, making a common pathophysiological mechanism likely.⁵ This hypothesis, highly suggested by early investigations which demonstrated 80-fold rise in Shiga-toxin-encoding gene expression with *E coli* O104:H4 and ciprofloxacin, was subsequently verified by the demonstration of phage induction and toxinosis caused by this same combination.^{5 13} Additional virulence factors in *E coli* O104:H4 include acquisition of up to three proteolytic molecules known as serine protease autotransporters of Enterobacteriaceae (SPATEs) and extended spectrum β -lactamases.⁵

Other chemotherapeutics prescribed for gastrointestinal upset are reported as possessing potential to worsen *E coli* infection. In the study of Wong *et al*¹⁴, antibiotic-treated children with *E coli* who developed HUS had received trimethoprim-sulfamethoxazole, amoxicillin or cephalosporins. The negative impact of sulphur-based drugs can be explained as they also activate the bacterial 'SOS' response and in vitro elicit comparable toxin release to ciprofloxacin.^{4 9} While β -lactams are not known to trigger this response, a mild experimental effect of ampicillin on

Figures 2 (A and B) Contrast CT showing bowel wall thickening (A—arrowed) and gross ascites and hypoperfused renal cortices (B—arrowed).

Figure 3 (A) Normalisation of platelet count in response to treatment. (B) Reduction of LDH in response to treatment. (C) Improvement of eGFR in response to treatment.

toxin levels has been demonstrated, which might be produced by cell wall lysis.⁶⁻⁹ Ciprofloxacin itself has previously been reported by Allan *et al*¹⁵ to induce HUS in a patient undergoing treatment for acute lymphoblastic leukaemia. However, despite the experimental evidence, until recently direct associations of a harmful impact in *E coli* patients were lacking. In 2011, a Danish study provided the first clinical link with four of five patients noted to have received initial empirical therapy with ciprofloxacin or metronidazole, another antibiotic which blocks DNA synthesis.⁶ Taken together with in vitro and animal studies, these cases and the patient we report suggest it may be preferable to avoid quinolones and other inhibitors of DNA synthesis when *E coli*, particularly O104:H4, is suspected.

Of necessity, all the experimental studies of ciprofloxacin toxicity in *E coli* infection employ subinhibitory concentrations as successful inhibition also blocks toxin production.⁹ As might not be unexpected in the case of drug-induced toxin release, the available evidence agrees with a clear dose-response relationship with a rise in toxin parallel to increase in ciprofloxacin from 1/16 to 1/2 of minimum inhibitory concentration.⁹⁻¹³ Indeed, ciprofloxacin at only 20% of clinical dosage was lethal to O157:H7-infected mice.⁴ Similar to the patient of Allan *et al*,¹⁵ our patient received a full therapeutic dose of

oral ciprofloxacin 500 mg BD. However, oral medication may be poorly taken up in gastrointestinal illness, preventing bactericidal action and facilitating toxin propagation, which is able to accelerate even if a substantial reduction in number of viable bacterial colonies is achieved.⁴⁻¹⁰ The attainment of minimum inhibitory concentration and therapeutic synergy, may be contributory factors to the recently announced beneficial effect of intravenous dual ciprofloxacin and meropenem therapy in the largest yet analysis of patients from the German outbreak, a retrospective case-control study of 298 patients, including 52, all from one centre, who received antibiotics.¹⁶ At that centre, oral rifamixin 600 mg once daily was also administered to the ITU patients and overall antibiotics were deemed to improve disease course, an interesting finding which now awaits validation through a randomised controlled trial.¹⁶⁻¹⁷ Of relevance to a possible action of therapeutic synergy, in vitro PVL studies suggest that toxin induction by subinhibitory levels of the β -lactam oxacillin could be negated if combined with the antibiotics rifampicin, linezolid or clindamycin, which had been shown to be capable of repressing PVL in culture.¹¹

Our patient's presentation with HUS 5 days after the onset of bloody diarrhoea matches the median time of 5 days from the first gastrointestinal disturbance to hospital

admission reported by Colic *et al.*⁶ While duration of antimicrobial therapy in the Danish cohort is not delineated, the short history suggests its initiation early in the illness course. This would be in line with the finding in the paediatric group of Wong *et al.*¹⁴ that patients given antibiotics within the first 3 days were at greater risk of HUS than those given antibiotics later in their disease course. In addition, both our case and the case of Allan *et al.*¹⁵ experienced notable worsening at 3 days after the start of ciprofloxacin therapy. These factors may help clinicians identify features of patient history consistent with *E coli*-associated HUS aggravated by chemotherapy and initiate appropriate management.

Advice to avoid quinolones in patients at risk of seizure is also of note in light of the high 30% rate of neurological complications, including delirium, decreased conscious level and seizures, documented in HUS associated with O104:H4.^{8 18} While the authors of the recent German report cite a reduced rate of seizure among HUS patients who received antibiotics, all these patients were treated by a single centre with a high preemptive use of antiepileptic drugs.¹⁶ Neurological manifestations make differentiation on clinical grounds of HUS from TTP, especially in adults, in whom TTP is the more common diagnosis, even more problematic. A 2009 Cochrane review did not recommend plasma exchange and FFP as superior to supportive care in HUS, in contrast to TTP.² Indeed, the German authors found equivocal benefit of plasma exchange and speculate a harmful effect of complement activation may be induced by the addition of FFP.¹⁶ However, patients from that cohort who did not receive plasma exchange had less severe illness than those who did.^{16 17} Our case and the Danish cohort—all of whom had neurological compromise⁶—responded well to this treatment approach. Unlike the Danish patients, we report response in a patient who required intubation and ITU admission, a possible indicator of disease severity, also reflected by the time to normalisation of platelet count, LDH and renal function (figures 3A–C, after the graphs in the paper of Colic *et al.*⁶). Authors of the Danish report have recommended randomised controlled trials for further investigation of the potential benefits of plasma exchange with FFP in HUS.⁶ Other options trialled in severe HUS include immunoabsorption, proposed to remove antibodies potentially causative of central nervous system symptoms⁸ and the complement-inhibitor eculizumab, although the new German report did not find a benefit of the latter.^{16 19}

Normal renal artery flow with poorly perfused renal cortices is unusual on CT and, in combination with oedematous bowel, is consistent with a toxin insult of gastrointestinal origin. In the context of history, examination and other supportive investigations, this may provide additional rationale for a toxin-related process. Recent release of clinical data on antibiotic-associated outcomes of patients from the 2011 outbreak has added complexity to our understanding of the influence of ciprofloxacin on O104:H4 disease course, as a possible benefit was elucidated of intravenous combination therapy with meropenem.¹⁶ However, empirical oral monotherapy, particularly with quinolones, may worsen toxin-mediated disease through the enhancing effect of subinhibitory drug concentrations on toxin release.

Learning points

- ▶ Quinolones and certain other classes of antibiotics, especially those which inhibit bacterial DNA synthesis, can exacerbate the clinical course in *Escherichia coli*-mediated haemolytic-uraemic syndrome (HUS), particularly with oral dosing.
- ▶ The capacity of quinolones to lower the seizure threshold may be relevant in light of the high rate of neurological complications in O104:H4-linked disease.
- ▶ Clinical utility of antibiotics not implicated in worsening of *E coli*-mediated HUS remains uncertain. In vitro data suggest carbapenems may be the most appropriate option if antibiotics are clinically necessary in a patient in whom *E coli* O104:H4 infection cannot be excluded. A newly published analysis of the German outbreak has identified the potential benefit of intravenous meropenem and ciprofloxacin with a third oral agent, rifamixin, added for patients in need of intensive therapy unit (ITU) admission. This approach now requires validation through a randomised controlled trial.
- ▶ Further research is required to inform the clinical debate over efficacy of plasma exchange and frozen plasma (FFP) in HUS as well as thrombotic thrombocytopenic purpura (TTP).
- ▶ CT findings of normal renal artery flow with poorly perfused renal cortices and oedematous bowel may provide supportive evidence for gastrointestinal toxin pathology.

Funding None.

Competing interests None.

Patient consent Obtained.

REFERENCES

1. Garg AX, Suri RS, Barrowman N, *et al.* Long term renal prognosis of diarrhea-associated haemolytic-uremic syndrome. A systematic review, meta-analysis and meta-regression. *JAMA* 2003;**290**:1360–70.
2. Michael M, Elliott EJ, Ridley GF, *et al.* Interventions for haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura. *Cochrane Database Syst Rev* 2009;(1):CD003595.
3. Health Protection Agency. *E. coli* O157 annual totals (on-line). 2012. http://www.hpa.org.uk/web/HPAweb&HPAwebStandard/HPAweb_C/1249113624846 (accessed 4 Jun 2012).
4. Zhang X, McDaniel AD, Wolf LE, *et al.* Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production and death in mice. *J Infect Dis* 2000;**181**:664–70.
5. Rasko DA, Webster DR, Sahl JW, *et al.* Origins of the *E. coli* strain causing an outbreak of haemolytic-uremic syndrome in Germany. *N Engl J Med* 2011;**365**:709–11.
6. Colic E, Dieperink H, Titledstad K, *et al.* Management of an acute outbreak of diarrhoea-associated haemolytic uraemic syndrome with early plasma exchange in adults from southern Denmark: an observational study. *Lancet* 2011;**378**:1089–93.
7. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *Lancet* 2005;**365**:1073–86.
8. Greinacher A, Friesicke S, Abel P, *et al.* Treatment of severe neurological deficits with IgG depletion through immunoabsorption in patients with *Escherichia coli* O104:H4-associated haemolytic uraemic syndrome: a prospective trial. *Lancet* 2011;**378**:1166–73.
9. McGannon CM, Fuller CA, Weiss AA. Different classes of antibiotics differentially influence Shiga toxin production. *Antimicrob Agents Chemother* 2010;**54**:3790–8.

10. **Kimmit PT**, Harwood CR, Barer MR. Induction of type 2 Shiga toxin synthesis in *Escherichia coli* O157 by 4-quinolones. *Lancet* 1999;**353**:1588–9.
11. **Dumitrescu O**, Badiou C, Bes M, *et al*. Effect of antibiotics, alone and in combination, on Pantone-Valentine leukocidin production by a *Staphylococcus aureus* reference strain. *Clin Microbiol Infect* 2008;**14**:384–8.
12. **Dumitrescu O**, Choudhury P, Boisset S, *et al*. β -lactams interfering with PBP1 induce Pantone-Valentine leukocidin expression by triggering *sa*A and *rot* global regulators of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2011;**55**:3261–71.
13. **Bielaszewska M**, Iidelevich EA, Zhang W, *et al*. Effects of antibiotics on Shiga- toxin 2 production and bacteriophage induction by epidemic *Escherichia coli* O104:H4 strain. *Antimicrob Agents Chemother* 2012;**56**:3277–82.
14. **Wong CS**, Jelacic S, Habeeb RL, *et al*. The risk of the haemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *N Engl J Med* 2000;**342**:1930–6.
15. **Allan DS**, Thompson CM, Barr RM, *et al*. Ciprofloxacin-associated haemolytic-uremic syndrome. *Ann Pharmacother* 2002;**36**:1000–2.
16. **Minne J**, Nitschke M, Stinge R, *et al*. Validation of treatment strategies for enterohaemorrhagic *Escherichia coli* O104:H4 induced haemolytic uraemic syndrome: case-control study. *BMJ* 2012;**345**:e4565.
17. **Artunc F**. Treating shiga toxin induced haemolytic uraemic syndrome. *BMJ* 2012;**345**:e4598.
18. **British National Formulary**. *BNF 63*. London: BMJ group and RPS publishing, 2012.
19. **Lapeyraqe AL**, Malina M, Fremeaux-Bacchi V, *et al*. Eculizumab in severe Shiga-toxin-associated HUS. *N Engl J Med* 2011;**364**:2561–3.

Copyright 2012 BMJ Publishing Group. All rights reserved. For permission to reuse any of this content visit <http://group.bmj.com/group/rights-licensing/permissions>.
BMJ Case Report Fellows may re-use this article for personal use and teaching without any further permission.

Please cite this article as follows (you will need to access the article online to obtain the date of publication).

Binks S, Regan K, Richenberg J, Chevassut T. Microbes without frontiers: severe haemolytic-uraemic syndrome due to *E coli* O104:H4. *BMJ Case Reports* 2012;10.1136/bcr-2012-006896, Published XXX

Become a Fellow of BMJ Case Reports today and you can:

- Submit as many cases as you like
- Enjoy fast sympathetic peer review and rapid publication of accepted articles
- Access all the published articles
- Re-use any of the published material for personal use and teaching without further permission

For information on Institutional Fellowships contact consortiasales@bmjgroup.com

Visit casereports.bmj.com for more articles like this and to become a Fellow