Exceptional Case

Cisplatin-induced haemolytic uraemic syndrome associated with a novel intronic mutation of CD46 treated with eculizumab

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Abstract
A 2-year-old patient with a neuroblastoma developed haemolytic uraemic syndrome (HUS) following treatment with cisplatin and carboplatin. Following treatment with eculizumab, there was a substantial improvement in renal function with the recovery of the platelet count and the cessation of haemolysis. Subsequent investigations showed a novel, heterozygous CD46 splice site mutation with reduced peripheral blood neutrophil CD46 expression. Withdrawal of eculizumab was followed by the recurrence of disease activity, which resolved with re-introduction of therapy. Abnormal regulation of complement may be associated with other cases of cisplatin-induced HUS and treatment with eculizumab may be appropriate for other affected individuals.

Keywords: CD46; cisplatin; eculizumab; haemolytic uraemic syndrome

Background

Haemolytic uraemic syndrome (HUS) is most frequently caused by infection with Shiga toxin-producing strains of Escherichia coli or Shigella dysenteriae type 1. Other causes include inherited and acquired abnormalities affecting the alternative pathway of complement, other infections and certain drugs [1], including cisplatin [2] and carboplatin [3]. Recently eculizumab, a humanized monoclonal antibody directed against C5 that prevents the activation of the terminal complement pathway, has been shown to be an effective treatment for patients with atypical HUS (aHUS) [4].

Thrombotic microangiopathy has long been recognized as a complication of disseminated cancer [5] and cisplatin therapy [6]. There are two patterns of presentation of cisplatin-associated HUS. Some patients present acutely, days after receiving the last dose of chemotherapy. Others present more insidiously with a latent period of up to 7 months [2]. The acute form has a high mortality rate, although successful treatment with plasma exchange has been described [2, 7, 8].

Case report
A previously well 27-month-old boy with no family history of kidney disease presented with a cervical mass.

Keywords: CD46; cisplatin; eculizumab; haemolytic uraemic syndrome

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ticulate material is characteristic of HUS (Figure 2).

105 days after the last dose. Before restarting the eculizumab, features suggested active HUS, and eculizumab was therefore recommenced with an initial dose of 600 mg given nightly doses of 300 mg according to the manufacturer’s dosing guidelines. Five weeks after administration of the first dose of eculizumab, the plasma creatinine concentration had reduced to 34 μmol/L, the plasma LDH was 532 iu/L, and the GFR was 67 ml/min/1.73 m². A decision was made to avoid further exposure to platinum-containing agents, and therapy was changed to a combination of vincristine, topotecan and doxorubicin. There was little further change in tumour size and, 126 days after starting chemotherapy, the tumour was surgically removed with clear excision margins and eculizumab was discontinued. At this time, the platelet count was 181 × 10⁹/L and the plasma creatinine peaked at 160 μmol/L and it steadily decreased thereafter (Figure 1). He also required progressively less blood product support. After a further dose of 300 mg a week later, he was maintained on fortnightly doses of 300 mg according to the manufacturer’s dosing guidelines. Five weeks after administration of the first dose of eculizumab, the plasma creatinine concentration had reduced to 34 μmol/L, the plasma LDH was 532 iu/L, and the GFR was 67 ml/min/1.73 m². A decision was made to avoid further exposure to platinum-containing agents, and therapy was changed to a combination of vincristine, topotecan and doxorubicin. There was little further change in tumour size and, 126 days after starting chemotherapy, the tumour was surgically removed with clear excision margins and eculizumab was discontinued. At this time, the platelet count was 181 × 10⁹/L and the plasma creatinine concentration was 34 μmol/L.

The platelet count fell to 138 four weeks after the last eculizumab dose and remained in the range of 98–160 × 10⁹/L for the next 2 months. The plasma creatinine remained stable for 6 weeks, and then rose to 44 μmol/L. Two months after discontinuing the eculizumab, the plasma LDH had risen to 807 iu/L. The serum haptoglobin was low at 0.4 g/L and rose to 0.96 g/L a week after the first dose. After 7 months of eculizumab therapy, the patient had normal haemoglobin (122 g/L), normal platelets (248 × 10⁹/L) and a plasma creatinine concentration of 32 μmol/L. The GFR had risen to 71 ml/min/1.73 m². The plasma concentration of sC5b-9 remained elevated at 133 ng/mL, casting doubt on the suitability of this assay for assessing activity and response to treatment.

DNA extracted from a buccal smear at the time of diagnosis of HUS was amplified by PCR for all coding exons ± 10 bases of the CFH (factor H), CFI (factor I), C3 (complement component 3), CFB (factor B) and CD46 (membrane cofactor protein, MCP) genes. Amplified products were subjected to bi-directional Sanger sequencing (ABI), as previously described [9]. CD46 expression on cell surfaces was assessed on peripheral blood neutrophils after recovery from myelo-suppression by flow cytometry using CD46 fluorescein isothiocyanate-conjugated antibody (B0 Pharmingen, 555949). An intronic sequence variant (c.1027+5G>T) was identified in CD46. This variant had not been previously reported nor was it detected in DNA from 188 normal control subjects. In silico splicing prediction analysis undertaken using Alamut (Interactive Bioscience Software, Seine Biopolis, 70 route de Lyons-la-foret, 76 000 Rouen, France; http://www.interactive-biosoftware.com) suggests that this variant may cause aberrant splicing. Flow cytometry analysis showed reduced CD46 expression on neutrophils compatible with haploinsufficiency. After discussion with the parents, a decision was made not to test other family members, because the result was felt to be of limited clinical utility, outweighed by potential anxiety induced in a carrier.

**Discussion**

There is evidence that cisplatin causes widespread endothelial damage [10]. Within the glomerulus, endothelial cells, glomerular basement membrane and podocytes all show ultrastructural evidence of damage after cisplatin exposure [11]. Opsonization of damaged or apoptotic cells with C3b plays an important role in the efficient removal of such cells by phagocytes [12], and there is evidence of complement activation in cisplatin-induced AKI. On host cell surfaces, deposited C3b is usually rapidly inactivated by several regulatory proteins including CD46 (MCP) and factor H, both of which act as cofactors for factor I. Deficiency or reduced activity of these complement regulatory proteins would potentially lead to amplification of the endothelial cell damage induced by cisplatin. This in turn would potentiate and maintain the thrombotic microangiopathy. aHUS is most frequently associated with impaired regulation of the alternate pathway of complement regulation on the surface of host cells with normal regulation in the fluid phase, and plasma C3 levels are therefore frequently normal [13]. Because endothelial damage is associated with complement activation [14] and C5 plays a pivotal role in the development of aHUS [15], it seemed appropriate to initiate treatment with eculizumab. The clinical condition of our patient improved rapidly after starting eculizumab. The finding of a novel mutation causing decreased expression of CD46 suggests that dysregulated complement activation played an important role in pathogenesis. The response to eculizumab, deterioration on withdrawal of eculizumab and improvement after restarting treatment all support this hypothesis. Our patient is...
Fig. 1. Graph summarizing key laboratory results. Thin arrows indicate doses of cisplatin; bold arrows indicate doses of eculizumab.
the first to have genetic investigation of the factors involved in the regulation of the alternative pathway of complement regulation and the first to be treated with eculizumab. The possibility that dysregulated complement activation underlies the thrombotic microangiopathy in other patients with cisplatin-induced HUS should be considered, and testing for abnormalities of complement regulation should be performed in future patients. Eculizumab therapy may be useful in other patients with cisplatin-induced HUS. The optimal duration of eculizumab therapy remains unclear; 4 months of therapy was clearly inadequate in our patient but we hope to be able to withdraw treatment in the future.

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