MONOCLONAL ANTIBODY THERAPY FOLLOWING LETHAL SHIGA TOXIN INJECTION IN MICE

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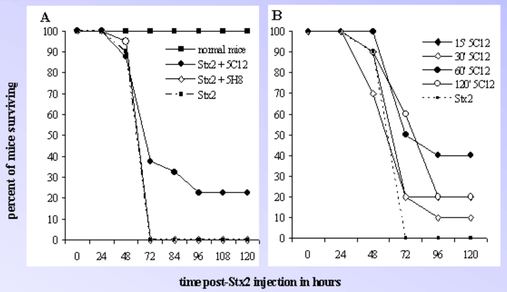
INTRODUCTION

Hemolytic uremic syndrome (HUS) occurs as a sequela of Shiga toxin (Stx)-producing *E. coli* (STEC) infection in children. Shiga toxins produced by STEC cross the gut mucosal barrier during acute STEC infection and disseminate systemically, initiating a constellation of symptoms that are collectively known as HUS: thrombocytopenia, microangiopathic hemolytic anemia, and acute renal failure. Severe complications of HUS include refractory renal failure, neurological impairment, and diabetes mellitus, which occur in a small subset of children with HUS. There is no effective treatment or prophylaxis for HUS, and thus current therapy is limited to supportive care. Passive administration of Stx-specific antibodies is a potential therapeutic intervention that could prevent or ameliorate HUS in STEC-infected patients. In pre-clinical trials, Stx2-specific human monoclonal antibody (HuMAb) 5C12 was effective at preventing organ-specific toxicity and lethality in animals when given within 48 hours of STEC infection; however, it is not known whether 5C12 can mitigate Stx2 toxicity when given after systemic dissemination of toxin. In the present study, mice were given an excessive lethal dose of Stx2 intravenously followed by specific HuMAbs (Stx2 A subunit-specific 5C12 or Stx2 B subunit-specific 5H8), also intravenously, at time intervals up to two hours after Stx2 injection. Mice dying of acute Stx2 intoxication showed clinical signs of uncompensated dehydration secondary to Stx2-mediated renal damage.

RESULTS



SURVIVAL: percent survival of mice given Stx2 intravenously (IV) and then Stx2 A and B subunit-specific human monoclonal antibodies 5C12 and 5H8, respectively, at different time points after Stx2. Overall, none of the mice receiving 5H8 ('B' subunit-specific, or binding, antibody) or Stx2 alone survived, whereas 22.5% of mice that received 5C12 antibody survived acute Stx2 toxicity (A). Survival was highest among mice that received antibody sixty minutes after Stx2 injection; however, this trend did not reach significance (B). Results suggest that 5C12 antibody binds Stx2 already bound to host cells and prevents further toxicity.

	Normal mice (n=6)	Convalescent mice (n=9)	5C12 acute toxicity	5H8 acute toxicity	Stx2 acute toxicity
Phosphorus (mg/dl)	10.8 ± 1.0	10.5 ± 1.0	**22.5**	**18.8**	**25.5**
Calcium (mg/dl)	11.3 ± 0.4	10.9 ± 0.4	**12.7**	**12.6**	**13.1**
Sodium (mEq/dl)	149 ± 3.1	153 ± 0.6	**145**	**139**	**136**
Potassium (mEq/L)	6.2 ± 0.7	6.8 ± 0.8	**7.2**	**9.0**	**5.8**
Na^+/K^+ (mg/L)	24 ± 2	23 ± 2	20	15	23

HEMATOLOGICAL AND URINARY PARAMETERS MEASURED: mean ± standard deviations are shown. In the acute phase of toxicity, mice became significantly dehydrated and urine was dilute as compared to control, normal mice. Mice that survived acute Stx2 toxicity showed persistent lack of urinary concentrating ability.

AZOTEMIA AND HEMOCONCENTRATION

	Normal Mice n = 6	Convalescent mice n = 9	5C12 acute toxicity	5H8 acute toxicity	Stx2 acute toxicity
Creatinine (mg/dl)	< 0.2	0.3 ± 0.1	**0.9**	**0.6**	**0.9**
Total protein (g/dl)	4.9 ± 0.5	5.2 ± 0.3	**8.6**	**8.5**	**7.9**
Albumin (g/dl)	3.2 ± 0.3	3.5 ± 0.2	**4.6**	**4.6**	**4.7**
Globulin (g/dl)	1.7± 0.2	1.7 ± 0.2	**4**	**3.9**	**3.2**
A/G ratio	1.9 ± 0.1	2.1 ± 0.3	1.2	1.2	1.5

PLASMA CHEMISTRY PROFILE: Severe protein and electrolyte abnormalities are seen in mice dying of acute Stx2 intoxication, regardless of antibody administration. Plasma samples from mice dying of acute Stx2 intoxication were pooled according to antibody administration in order to obtain sufficient volume for analysis. Mice dying of acute Stx2 intoxication show signs of uremia characterized by severe hyponatremic dehydration, decreased glomerular filtration rate, renal azotemia, and hyperglycemia.

PLASMA ELECTROLYTES

	Normal mice (n=6)	Convalescent mice (n=9)	5C12 acute toxicity	5H8 acute toxicity	Stx2 acute toxicity
Phosphorus (mg/dl)	10.8 ± 1.0	10.5 ± 1.0	**22.5**	**18.8**	**25.5**
Calcium (mg/dl)	11.3 ± 0.4	10.9 ± 0.4	**12.7**	**12.6**	**13.1**
Sodium (mEq/dl)	149 ± 3.1	153 ± 0.6	**145**	**139**	**136**
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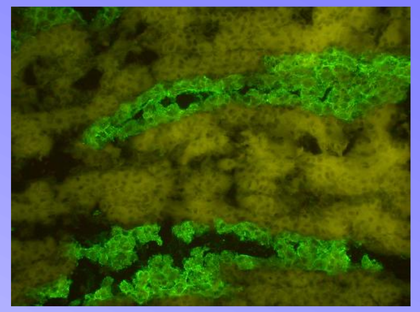
HISTOLOGY



CONCLUSIONS

- Antibodies targeted against the enzymatically active 'A' subunit (5C12), but not the binding 'B' subunit (5H8), of Stx2, improved survival among mice when administered up to two hours after Stx2.
- Mice dying of acute Stx2 intoxication showed signs of uremia characterized by severe hyponatremic dehydration, decreased glomerular filtration rate, renal azotemia, and hyperglycemia. Clinical signs and histological data suggest Stx2 intoxication leads to loss of renal tubular function.
- Mice surviving acute Stx2 intoxication showed persistently low urine specific gravity measurements, suggesting that 5C12 can mitigate, but not prevent, Stx2-mediated nephrotoxicity when administered up to 2 hours after lethal doses of Stx2.
- The results of this experiment indicate that administration of 5C12 antibody can prevent further progression of primary renal toxicity when administered following systemic dissemination of Stx2, thus protecting some mice from death. While additional studies are required to fully characterize the protective effects of 5C12 following systemic dissemination of Stx2, this experiment further supports the potential use of 5C12 monoclonal antibody in patients presenting with HUS.

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