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BRAIN RESEARCH

Research Report

Intracerebroventricular administration of Shiga toxin type 2 induces striatal neuronal death and glial alterations: An ultrastructural study

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ABSTRACT

Shiga toxin (Stx) from enterohemorrhagic *Escherichia coli* (STEC) is the main cause of hemorrhagic colitis which may derive to hemolytic–uremic syndrome (HUS). HUS is characterized by acute renal failure, thrombocytopenia and microangiopathic hemolytic anemia. Mortality in the acute stage has been lower than 5% of total affected argentine children with endemic HUS. Common signs of severe CNS involvement leading to death included seizures, alteration of consciousness, hemiparesis, visual disturbances, and brainstem symptoms. The main purpose of the present work was to study the direct involvement of Stx2 in brain cells by intracerebroventricular (i.c.v.) administration of Stx2. Immunodetection of Stx2 was confirmed by immunoelectron cytochemistry in different subsets and compartments of affected caudate putamen cells of corpus striatum. Transmission electron microscopy (TEM) studies revealed apoptotic neurons, glial ultrastructural alterations and demyelinated fibers. The i.c.v. microinfusion was applied for the first time in rats to demonstrate the direct action of Stx2 in neurons and glial cells. The toxin may affect brain neuroglial cells without the involvement of proinflammatory or systemic neurotoxic elements.

1. Introduction

Shiga toxin (Stx) from enterohemorrhagic *Escherichia coli* (STEC) is the main cause of hemorrhagic colitis which may derive to hemolytic–uremic syndrome (HUS) (O'Brien and Kaper, 1998), a triad of events which include: thrombocytopenia, microangiopathic hemolytic anemia, and acute renal failure (Proulx et al., 2001). Argentina is the first country with 400 new cases a year. Mortality in the acute stage has been lower than 5% of total affected argentine children with endemic HUS since 1978 (Repetto, 2005). Children usually die because of severe involvement of the central nervous system (Exeni, 2001; Eriksson et al., 2001; Oakes et al., 2006). Common signs of severe CNS involvement included seizures, alteration of consciousness, hemiparesis,

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Abbreviations: HUS, hemolytic–uremic syndrome; i.c.v., intracerebroventricular; Stx, Shiga toxin; STEC, Shiga toxin from enterohemorrhagic *Escherichia coli*; TEM, transmission electron microscopy

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visual disturbances and brainstem symptoms (Siegler, 1994; Gallo and Gianantonio, 1995). Some authors suggested that the origin of CNS pathogenesis by HUS was secondary to metabolic changes: hyponatraemia, azotaemia, hydration disorders or hypertension (Siegler, 1994; Gallo and Gianantonio, 1995). Other authors claimed more for infarction of the brain microvasculature by Stx leading to neurological damage (Taylor et al., 1999; Mizuguchi et al., 2001). And whether Stx2 directly affected brain neuroglial cells is yet to be investigated. To study this issue then the toxin should not pass through brain microvasculature, through the blood–brain barrier (BBB). One way to circumvent the microvasculature is to i.c.v. microinfuse the toxin in a certain brain area. Therefore, does Stx2 possess a direct neurotoxic involvement on brain parenchymal cells? This prompted us to study the role of Stx2 on brain injury.

The studies of brain intoxication produced by Stx were usually performed in animal models of STEC colonic invasion, Stx intraperitoneal (i.p.) or endovenous (e.v.) systemic administrations. In some previous reports, the action of Stx2 in brain was focused on mitomycin-treated mice intragastrically inoculated with the *Escherichia coli* O157:H– strain E32511/HSC (Fujii et al., 1994). In addition, studies with Stx2 administration on animal models for the toxin neuropathogenicity was only performed by e.v. (Fujii et al., 1996; Yamada et al., 1999; Mizuguchi et al., 2001) or intrathecal (Mizuguchi et al., 1996, Fujii et al., 1998) toxin administration in rabbit brains (Fujii et al., 1996, 1998; Mizuguchi et al., 1996, 2001; Yamada et al., 1999). E.v. administration caused a selective damage of neurons seen in the lower layers of the cerebellar and cerebral cortex, midbrain and spinal cord and in a later phase involvement of pathological changes of blood vessels (Mizuguchi et al., 1996). However, immunodetection of the toxin was not shown in the brain parenchyma; it was rather observed on blood vessels' walls. Other MRI studies performed in rabbits showed brain lesions in the hypothalamus, hippocampus, brain stem, medulla (Fujii et al., 1996) and cerebellum after e.v. Stx2 or intrathecal injection (Fujii et al., 1998). Although this technique proved to be an efficient tool to detect brain lesions, the topographic distribution of the toxin within an affected brain area and its toxic influence at the cell level could not be determined. This technique therefore is not suitable to observe the action of Stx at the cell level.

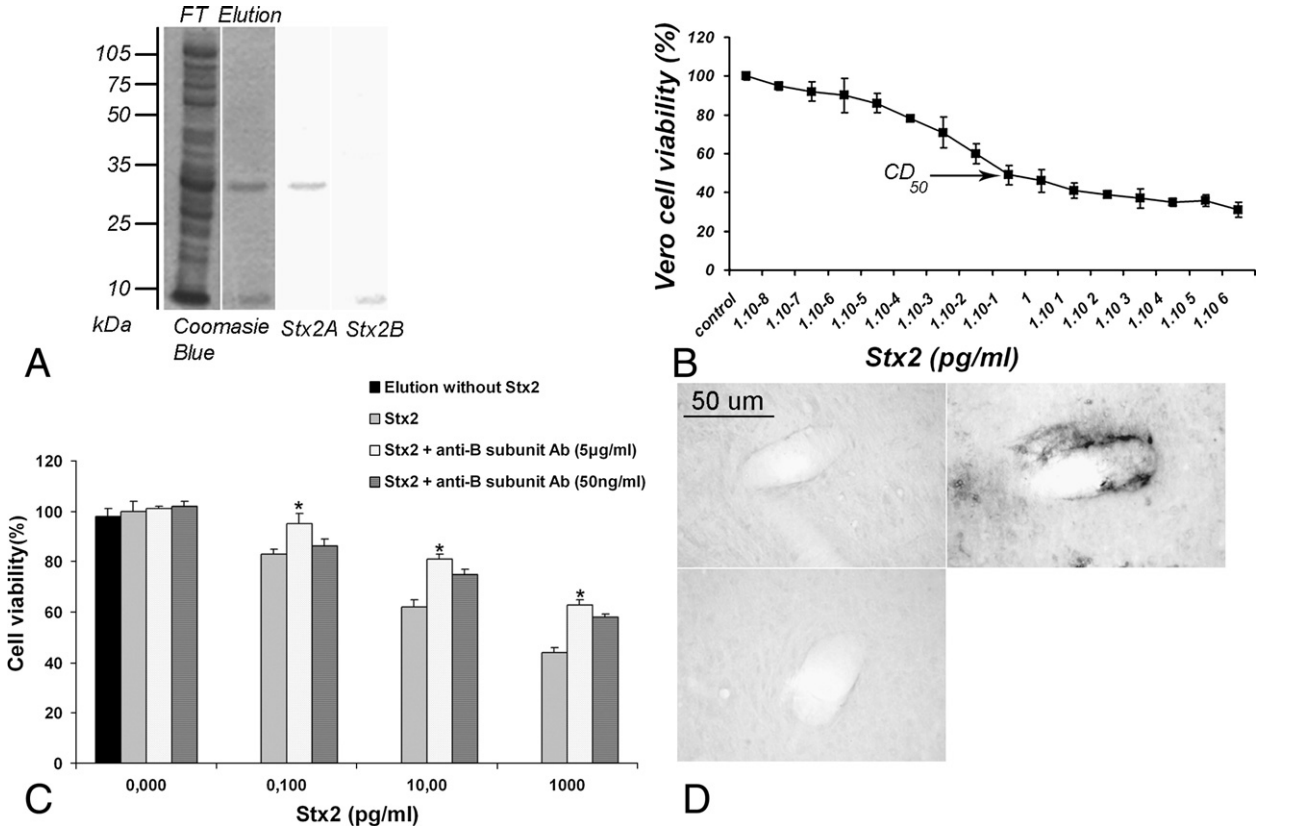

Fig. 1 – Stx2 obtained by affinity chromatography purification was cytotoxic to Vero cells and passed the blood–brain barrier (BBB). An elution that contained Stx2 was analyzed on a SDS–PAGE electrophoretic gel revealing two bands that corresponded to the A and B subunits of Stx2 on Coomassie blue staining (A); these bands were confirmed by WB (A). (FT: flow through). The Stx2 cytotoxic capacity was confirmed on a Vero monolayer cell culture (B). Preincubation of the toxin with increasing concentrations of the monoclonal anti-Stx2B antibody resulted in a significant increase in Vero cell viability in a dose–response manner (C). In B and C, data are reported as means ± S.E.M. of at least three triplicate experiments. *$P<0.05$ (ANOVA). Intraperitoneal Stx2 administration showed that the toxin was able to pass the blood–brain barrier (D); Stx2 immunolocalization was confined to parenchymal brain cells near perivascular spaces (right panel). In the above left panel (D) the isotype control shows non-specific immunoreaction, while a negative control is shown by omitting the primary antibody in the below panel.