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24 *Current Neuropharmacology*, **2021**, *19*, 24-44

REVIEW ARTICLE

Therapeutic Strategies to Protect the Central Nervous System against Shiga Toxin from Enterohemorrhagic *Escherichia coli*

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ARTICLE HISTORY

Received: January 15, 2020
Revised: January 20, 2020
Accepted: February 19, 2020

DOI:
10.2174/1570159X18666200220143001

Abstract: Infection with Shiga toxin-producing *Escherichia coli* (STEC) may cause hemorrhagic colitis, hemolytic uremic syndrome (HUS) and encephalopathy. The mortality rate derived from HUS adds up to 5% of the cases, and up to 40% when the central nervous system (CNS) is involved. In addition to the well-known deleterious effect of Stx, the gram-negative STEC releases lipopolysaccharides (LPS) and may induce a variety of inflammatory responses when released in the gut. Common clinical signs of severe CNS injury include sensorimotor, cognitive, emotional and/or autonomic alterations. In the last few years, a number of drugs have been experimentally employed to establish the pathogenesis of, prevent or treat CNS injury by STEC. The strategies in these approaches focus on: 1) inhibition of Stx production and release by STEC, 2) inhibition of Stx bloodstream transport, 3) inhibition of Stx entry into the CNS parenchyma, 4) blockade of deleterious Stx action in neural cells, and 5) inhibition of immune system activation and CNS inflammation. Fast diagnosis of STEC infection, as well as the establishment of early CNS biomarkers of damage, may be determinants of adequate neuropharmacological treatment in time.

Keywords: Neurodegeneration, neuroprotection, neuropharmacology, reactive astrocytes, microvasculature, oligodendrocytes, microglial cells, Shiga toxin 2, images, brain, cerebellum, transmission electron microscopy, fluorescence microscopy, lipopolysaccharides, inflammation, Hemolytic Uremic Syndrome.

1. INTRODUCTION

Shiga-like toxins (Stx), also known as verotoxins [1], are a virulence factor released by enterohemorrhagic *E. coli* (EHEC) and non-EHEC bacteria, both generically called Shiga-toxin-producing *E. coli* (STEC) [2, 3].

Shiga toxins belong to an AB family of bacterial toxins, which includes tetanus (from *Clostridium tetani*), cholera (from *Vibrio cholerae*), anthrax (from *Bacillus anthracis*) and diphtheria toxins (from *Corynebacterium diphtheriae*), among others [4]. AB toxins are named after their two basic protein components: the catalytic A component exerts its action in intracellular molecules, while the B component is responsible for the binding of the toxin to specific receptors on target cells [4, 5]. In the case of Stx (Fig. **1**), the B subunit is a homopentamer-structure that recognizes the receptor globotriaosylceramide (Gb3).

The interaction between Stx and Gb3 is responsible for the hemolytic-uremic syndrome (HUS), a clinical syndrome which primarily targets children up to 5 years of age [6]. HUS is a triad characterized by thrombocytopenia, microangiopathic hemolytic anemia and variable degrees of renal compromise, ranging from minor urine abnormalities to severe renal disease, which may be preceded by prodromal bloody diarrhea in STEC-infected patients [6-11]. STEC causes more than 2.8 million annual acute illnesses worldwide, leading to 3890 cases of HUS and 230 deaths [12]. The central nervous system (CNS) is frequently affected, producing an acute encephalopathy which is responsible for a worse prognosis. The mortality rate derived from HUS adds up to 5% of the cases, and up to 40% when the CNS is involved [13].

Stx genes are encoded within a chromosomally integrated lambdoid prophage genome [14-16]. Antibiotics promote Shiga toxin production by inducing the lithic cycle of the lambda bacteriophage, thus enhancing the replication and expression of Stx genes. For these reasons, the use of conventional antibiotics is contraindicated and even increases the risk of developing HUS [17]. At present, there is no consensus high-efficiency treatment for STEC infections [18]. In addition to Stx, STEC also releases lipopolysaccharides (LPS), the main component of the outer membrane of Gram-negative bacteria responsible for serogroup identification (O antigen) [5]. The frequent endotoxemia observed in HUS patients suggests an important role of LPS in STEC pathogenesis [19].

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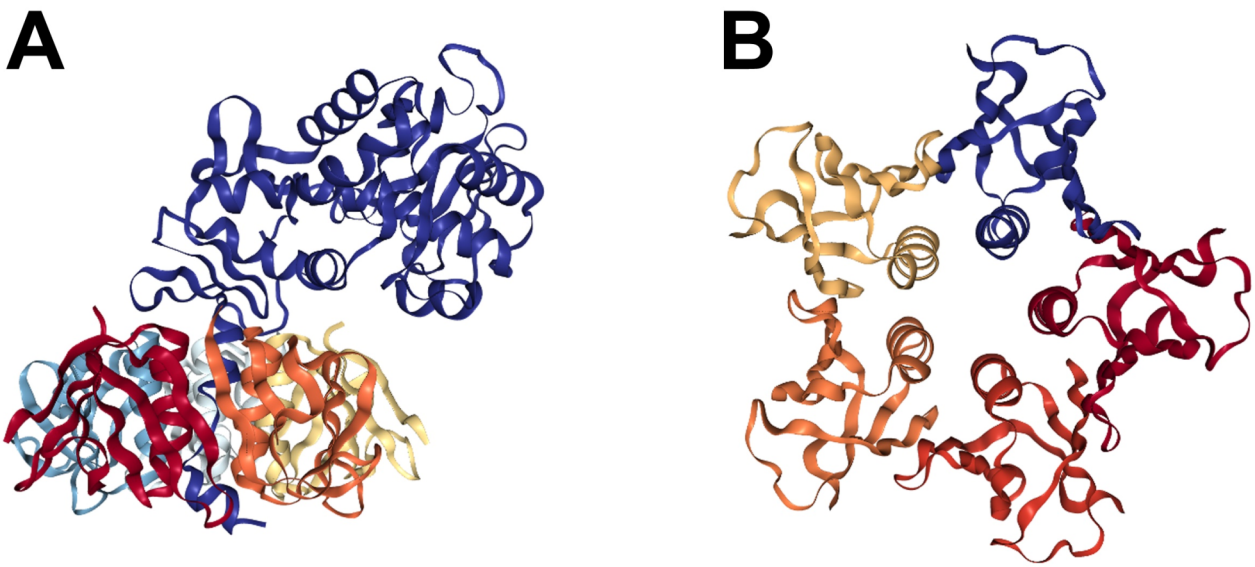

Fig. (1). Crystal structure of Shiga toxin. A: crystal structure of Shiga toxin. The A-subunit is shown in dark blue and the B-subunit is shown in different colors (PDB #1R4P); **B**: Shiga toxin B-subunit with individual B-monomers is shown in different colors (PDB #3MXG); PDB images were obtained from Research Collaboratory for Structural Bioinformatics Protein Data Base (www.rcsb.org). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

STEC major reservoir is cattle; therefore, human infection is produced through the consumption of contaminated bovine-derived products, contaminated water, unpasteurized apple drinks and vegetables. STEC has a low infective dose; only 10 colony-forming units can produce an infection in humans, which allows potential direct transmission through contact with infected people or animals [20-23].

2. STX MECHANISM OF ACTION

Based on their serological neutralization profile, there are two main groups of Stx: Stx1, which has a molecular mass of 70 kDa, and Stx2, which has 60 kDa [24]. Each group, in turn, comprises various subtypes named in letters [25]. Stx1 shows remarkable homology with Stx from *Shigella dysenteriae*. Furthermore, it can be neutralized by antibodies raised against Stx, as they differ in only one amino acid residue. On the other hand, Stx2 shows around 55 % homology with *Shigella´*s toxin and cannot be neutralized by *Shigella*-raised antibodies [26]. Although the affinity of Stx1 to its receptor is 10-fold higher than that of Stx2, the latter has 400-fold higher toxicity than Stx1 in mice [27] and is primarily responsible for severe cases in human infections [28-31].

The B subunit of Stx is a homopentameric protein non-covalently associated with the A subunit by its terminal carboxyl extreme [4, 32]. The B homopentamer binds to the membrane receptor globotriaosylceramide (Gb3), a glycosphingolipid present on a detergent-insoluble portion of lipid raft membranes rich in cholesterol [33].

Once the interaction of StxB-Gb3 occurs, it evokes an endocytic process which may be clathrin-dependent or independent [34, 35] and which culminates in a retrograde pathway that ends in the cytosol. Early endosomes containing Stx escape the lysosomal pathway to the trans-Golgi network and the Golgi apparatus to finally reach the endoplasmic reticulum. The A subunit contains a loop formed by a disulfide bond involving two cysteines in positions 242 and 261. This loop area contains the sequence Arg-X-X-Arg, which is enzymatically cleaved by furin in two different chains: A1, which has a molecular mass of 27.5kDa, and A2, with a molecular mass of A2 4.5 kDa [33, 36]. These two chains remain linked by a disulfide bond. The A1 chain –which contains the catalytic N-glycosidase activity–subsequently translocates to the cell cytosol and removes the adenine residue 2260 of 28S eukaryotic rRNA. Thus, protein synthesis is inhibited at the translational level as the elongation factor EIF2a no longer binds to ribosomes [37-39], which triggers a ribotoxic stress response of pro-apoptotic and pro-inflammatory events [27, 40] (Fig. **1**).

Gb3 is heterogeneously distributed in the body. It may be found in endothelial cells, hematopoietic cells, pancreas, heart, liver, kidney and the CNS [8]. Although the Gb3 biological function has not been entirely elucidated yet, recent evidence suggests that it plays an essential role in protein reabsorption by renal proximal tubules [41]. Furthermore, Gb3 is the Pk antigen from the human P blood group system [42, 43] and the CD77 antigen associated to Burkitt's lymphoma [44]; it is also present in activated B-cells which undergo apoptosis after not being selected for plasma ce11 differentiation in germinal centers of lymphoid tissue [45].

Gb3 consists of a ceramide moiety composed of different fatty acid chains linked by an amide bond to a sphingosine, which, in turn, is linked to a sugar chain (galactose α 1-4 galactose β 1-4 glucose). The ceramide element is composed by relatively constant sphingosine and a highly variable fatty acid chain which, depending on the cell type and the stage of the cell cycle, may show different lengths and degrees of saturation [46]. Therefore, there are many Gb3 isoforms whose fatty acid chain length and saturation degree influence the cytotoxic action of Stx, with long fatty acid chains being responsible for greater toxicity. The amount of cholesterol

and phosphatidylcholine seems to be an important factor responsible for toxin internalization [4, 47, 48]. It has been reported that cholesterol microdomains enhance the binding and the entry of Stx [4], probably because cholesterol can modulate the orientation of the carbohydrate group of Gb3 [48].

Polymorphonuclear leukocytes (PMN), which do not express Gb3, have been reported to be the main carriers of Stx from the intestine to systemic organs [49-53]. Stx binds to PMN *via* the TLR4 receptor, which has a 100-fold lower affinity than the Gb3 receptor; however, pre-treatment with LPS induces a 30-fold increase in specific binding sites for Stx on PMN [54].

Another non-canonical way in which Stx may act is through a delivery system in Stx-containing microvesicles from various types of cells. These microvesicles charged with Stx also have the intrinsic ability to induce thrombosis by activating coagulation factors, as they contain the activated complement components phosphatidylserine and tissue factor [55]. Free Stx in the bloodstream is almost undetectable, as it is either attached to blood cells or present in Stx-containing microvesicles which have a cytotoxic effect equivalent to that of the free toxin.

Stx reaches the cerebral parenchyma by breaking the blood-brain barrier (BBB) [56]. However, alternative cerebral parenchyma routes of access should not be excluded, such as the blood-cerebrospinal fluid (CSF) barrier and circumventricular organs, *i.e*., structures located around the third and fourth ventricles characterized by a lack of the BBB.

Stx may also exert an important cytotoxic effect by inducing a strong inflammatory status. This is achieved through the activation of leukocytes, endothelial cells and the alternative pathway of complement, with the consequent production of reactive oxygen species (ROS) and the release of cytokines/chemokines. This secondary effect may be heightened by LPS and it may contribute to the physiopathology of the disease [57]. Furthermore, pro-inflammatory cytokines induced by LPS, such as TNF-α, promote the upregulation of Gb3, which increases cellular sensitivity to Stx [56, 58]. Clinical evidence supporting these events was found in patients with encephalopathy-derived STEC infections with elevated concentration of plasma TNF-α, a soluble form of types I and II TNF receptor, neopterin, IL-8 and IL-6 [59, 60]. It has also been shown that LPS non-responder mice treated with Stx2, together with LPS, have mild systemic symptoms with later isolated neurologic symptoms, in contrast with LPS responder mice, which develop a severe combination of gastrointestinal, neurologic and systemic symptoms. This indicates that the combination of Stx and LPS is a determinant contributor to the pathogenesis of the disease [61].

3. STX AND THE HUMAN CNS

The abnormal accumulation of metabolic products in circulation due to renal failure includes toxic compounds like creatinine and uric acid, among others, which may produce uremic encephalopathy leading to cognitive dysfunction, motor disorders and seizures [62]. During HUS, kidney damage correlates with an increase in these metabolites, although up to 15% of the patients positively diagnosed with STEC develop encephalopathy before the onset of HUS. This event supports the hypothesis that the cerebral damage in STEC infections may be produced directly by Stx in the neural tissue and not by a mere accumulation of metabolic products due to renal failure [63].

In humans, CNS symptoms of this disease are diverse, with patients suffering from blindness, hyperreflexia, deficits in orientation, attention or memory abilities, poor fine-motor coordination, seizures or irregular myoclonus and coma, among others [64-76]. Brain magnetic resonance images have also shown recurrent edema with hemorrhagic components, which indicates the presence of inflammation and vascular damage. These changes were observed in distinct brain areas such as basal ganglion, thalamus, midbrain, corpus callosum, cerebellum, white matter and brain stem [64-66, 68-73]. Furthermore, CSF analyses have shown an elevated protein concentration in 10 to 30% of patients. This fact is consistent with BBB disruption, as protein concentration in normal CSF is low compared to serum [67, 77].

In addition, post-mortem samples have shown edema and focal infarcts due to hypoxic/anoxic/ischemic events [77]. Focal microhemorrhages are often found together with hypoxic-ischemic changes throughout the brain. Although the pathophysiology of HUS involves small vessel occlusions, cerebral occlusive syndromes are not frequently reported. Very few histopathological findings have reported micro thrombosis in the CNS, which suggests the involvement of endothelial damage with no significant platelet or coagulation activation [73, 78].

In May 2011, an outbreak of STEC was reported in northern Germany, with a total of 53 associated deaths. According to the final report of the Robert-Koch-Institute [71], a total of 3842 patients were infected with STEC O104:H4, while 855 patients developed HUS and exhibited neurological symptoms in the seventh day after the onset of diarrhea. Neurological symptoms in these cases included double vision, difficulties in finding words, hyperreflexia, deficits in orientation, attention or memory abilities and seizures, among others. Almost 29 % of those patients needed mechanical ventilation in the course of the disease due to severe alteration of consciousness. Furthermore, the severity of encephalopathy is usually accompanied by increased pro-inflammatory TNF-α and IL-1β cytokines, which suggests an important role of cytokines in the pathogenesis of the disease. Infection by STEC leads to the production of these cytokines not only in the intestine, but also in systemic circulation and in the brain [70]. In addition, in 2019, the Center for Disease, Control and Prevention (CDC) reported five outbreaks of STEC infection triggered by the intake of the following contaminated foods: fresh express sunflower crisp chopped salad kits, romaine lettuce, ground bison, flour and ground beef (https://www.cdc.gov/ecoli/2019/o103-04-19/index.html)

4. PHARMACOLOGY

Although conservative therapy appears to be a well-accepted treatment for HUS patients, no consensus therapy

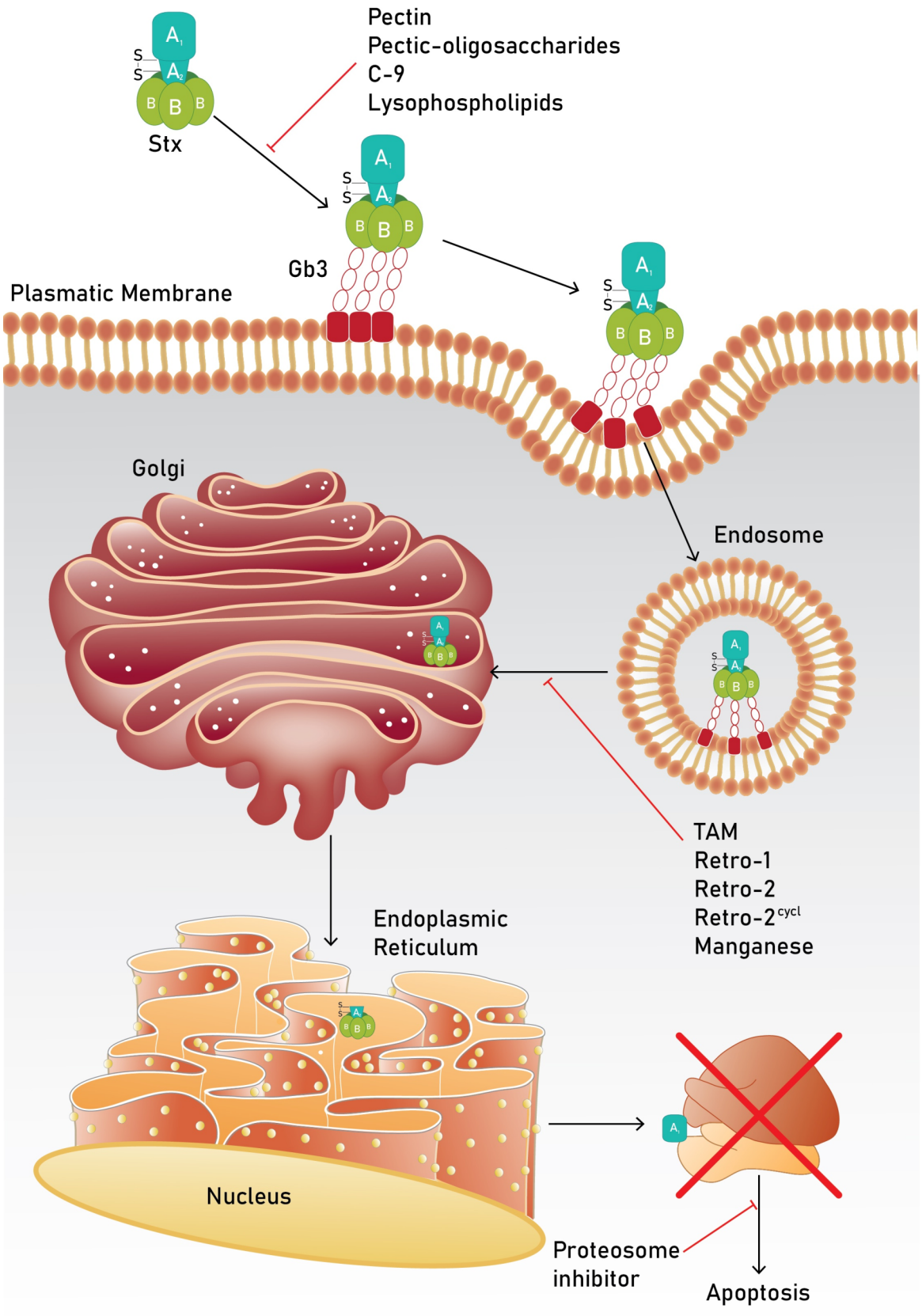

Fig. (2). Stx cellular pathway, action and therapeutic strategies to block it. Black arrows show Stx cellular pathway and action: binding of Stx to Gb3, endocytosis process, the retrograde pathway to trans-Golgi network and endoplasmic reticulum. A1 subunit reaches cytosolic ribosomes, inhibits protein synthesis and leads to apoptosis. Red blunt arrows show specific Stx pathway and action blocked by each therapeutic approach. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

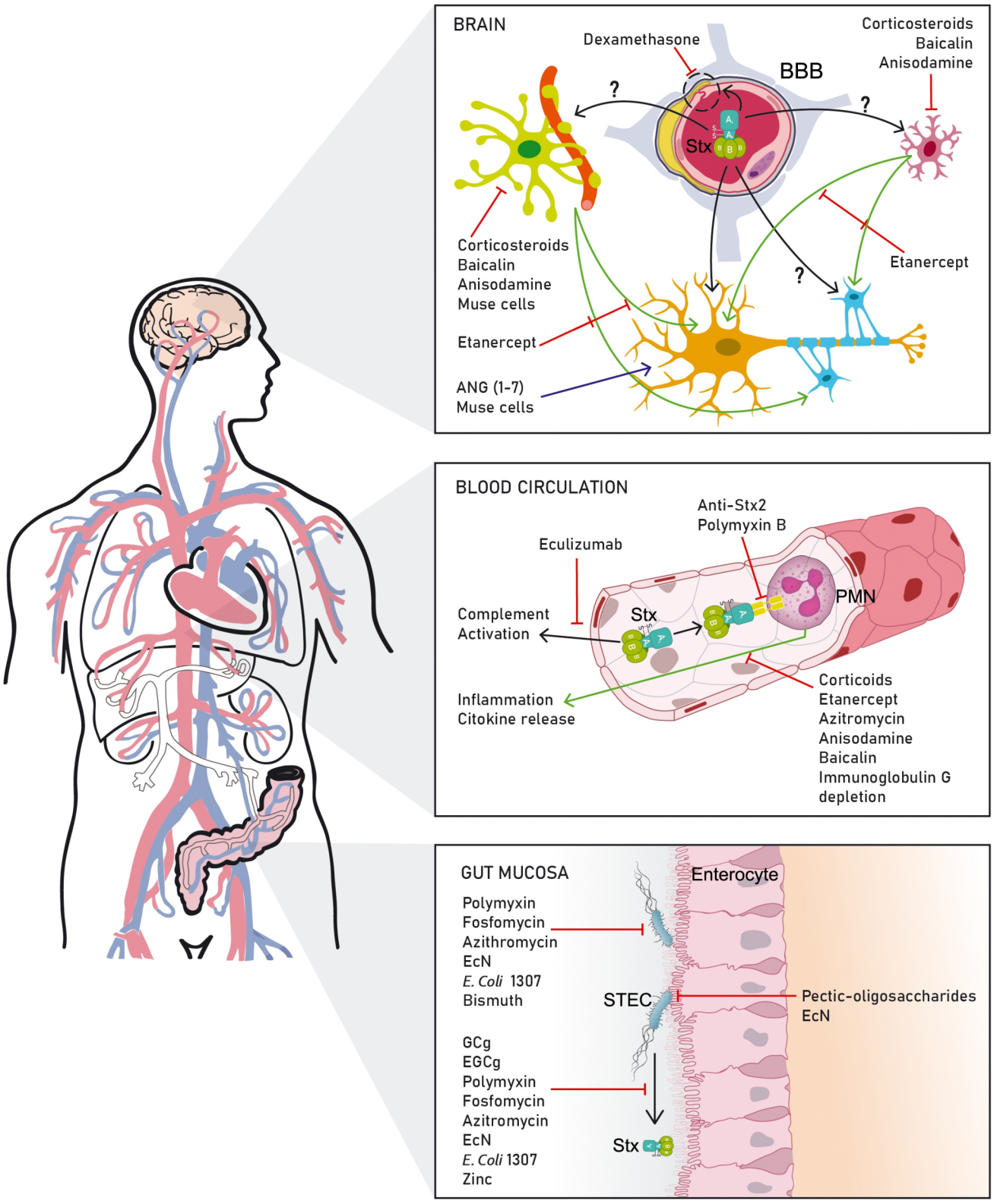

Fig. (3). Therapeutic strategies to protect the CNS against Shiga toxin. Black arrows show the consequences of STEC infection and the direct effects of Stx in the body. Question mark on black arrows suggests a cellular effect of Stx not described yet. Green arrows show the cell release of deleterious factors in response to Stx. Red blunt arrows show specific Stx pathway and action blocked by each therapeutic approach or an inhibitory effect of these drugs on STEC bacteria in the gut mucosa. Blue arrow shows drugs that produce their neuropharmacological action without blocking any specific Stx pathway or action. Green cell: astrocyte in contact with a vessel; red cell: microglia; blue cell: oligodendrocyte; orange cell: neuron. BBB: blood-brain-barrier; PMN: polymorphonuclear. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 1. Brief description of the drugs analyzed in this review.

Drug Class	Drug	Type of Study	Effect	Refs.
Antibiotics	Polymyxin B	*In vitro* (human neutrophils)	Inhibited the interacion of StxA with TLR4.	Carnicelli *et al*., 2016 [76]
			Inhibition of neutrophils activation.	
	Polymyxin E (Colistin)	*In vitro* (*E. coli* O157:H7)	Reduced in a dose-dependent manerthe release of Stx2 and LPS.	Percivalle *et al*., 2016 [83]
	Fosfomycin	Clinical study	Protected patients against Stx	Ikeda *et al*., 1999 [90]
	Azythromycin	*In vitro* (*E. coli* O86:H-)	Had a low MIC and inhibited Stx production	Ohara *et al*., 2002 [92]
		In vitro (Human mononuclear cells)	Inhibited the Stx1/Stx2-stimulated cytokine production	
		In vivo (murine model)	Decreased in Stx-induced proinflamatory citokynes production	
			Protected effect against Stx challenge	
Anti-inflammatory	Betamethasone	*In vivo* (rabbit model)	Reduced rabbit mortality	Fujii *et al*., 2009 [102]
			Protected rabbit against brain edema	
	Methylprednisolone	Case report	Reduced systemic proinflammatory cytokines	Oki *et al*., 2008 [104]
		Case roport	Patient recovered without any sequela	Yoshimitsu *et al*., 2011 [105]
		Case report	Improved the patient condition	Shimizu *et al*., 2014 [57]
		Clinical study	Patients recovered completely	Takanashi *et al*., 2014 [103]
		Case report	Patient condition improved gradually	Ito *et al*., 2015 [56]
		Case report	Improved the patient condition	Yada *et al*., 2015 [106]
		Clinical study	Increased the patients good outcome	Kuroda *et al*., 2015 [107]
		Case report	Patient recover without any sequela	Hosaka *et al*., 2017 [91]
	Dexamethasone	*In vivo* (murine model)	increased the survival of mice challenged with a lethal doses of Stx2	
			Protected neuronal populations present in different brain regions	Pinto *et al*., 2013 [112]
			Reduced astrocyte/microglial reaction & damage to the myelin sheath	Pinto *et al*., 2017 [53]
			Protected the BBB & restored the basal expression of VEGF	Pinto *et al*., 2018 [113]
			Reversed changes in mice behavior	
	Etanercept	*In vivo* (rat model)	Reduced the Stx2 uptake by neurons & its lethal effect	Pinto *et al*., 2018 [113]
Vasoactive drugs	Angiotensin 1-7	*In vitro* (mixed glia mouse culture)	Did not prevent oligodendrocyte damage	Goldstein *et al*., 2016 [118]
		In vivo (rat model)	Prevented Stx2-induced damage in neurons and oligodendrocytes	
	Anisodamine	*In vitro* (Human monocytic cells)	Inhibited the production of TNF-α, IL-1β and IL-8	Zhang *et al*., 2000 [135]
		In vivo (murine model)	Increased the survival of Stx1-treated mice	
Antibodies	Eculizumab	Clinical study	Improved the patient condition rapidly	Lapeyraque *et al*., 2011 [147]
		Clinical study	Produced a good neurological outcome	Gitiaux *et al*., 2013 [145]
		Case report	Produced an improvement of the patiant neurologic status	Saini *et al*., 2015 [149]
		Clinical study	Produced a good neurological outcome	Pape *et al*., 2015 [65]
		Review	Produced a positive improvement in patient condition	Mahat *et al*., 2019 [148]

(Table 1) contd….

Drug Class	Drug	Type of Study	Effect	Refs.
Antibodies	Antibodys anti-Stx	*In vivo* (murine model)	Protected mice challenged with a lethal charge of STEC and from Stx	Yamagami *et al*., 2001 [160]; Kimura *et al*., 2002 [158]
		In vitro (ACHN cells)	Protected cells against Stx	Kimura *et al*., 2002 [158]
		In vivo (murine model)	Prevented the lethal effects of Stx	Santer *et al*., 2008 [161]
		In vivo (murine model)	Protected mice challenged with Stx	Mejias *et al*., 2016 [157]
		In vitro (Vero cells)	Protected cells against Stx	
		Phase 1 safety and pharmacokinetic study	Were well tolerated by patients	Dowling 2005 [162]; Bitzan 2009 [163]; Lopez 2010 [164]
	Stx vaccine	*In vivo* (murine model)	Protected mice challenge with a lehtal dose of Stx2 or lethal charge with EHEC	Mejias *et al*., 2013 [166]; Mejias *et al*., 2014 [165]
Polyphenols	Polyphenols	*In vitro* (Vero cells)	Protected cells against Stx	Quinones *et al*., 2009 [172]
	Baicalin	*In vitro* (Vero cells)	Protected cells against Stx	Vinh *et al*., 2019 [176]
		In vitro (HELA cells)	Protected cells against Stx	Dong *et al*., 2015 [174]; Zhang *et al*., 2017 [175]
		In vivo (mice)	Protected mice challenged with Stx	Dong *et al*., 2015 [177]
		In vivo (mice)	Protected mice challenged with a lethal charge of E. coli O157:H7	Zhang *et al*., 2017 [175]
	Catechins	*In vitro* (*E. coli* O157:H7)	inhibited bacteria growth and suppressed the release of Stx from STEC	Sugita-Konishi *et al*., 1999 [177]
Pharmabiotics	prebiotics	*In vitro* (Human HT29 cells)	Protected cells against Stx	Olano-Martin *et al*., 2003 [189]
		In vitro (Human HT29 cells)	Inhibited *E. coli* O157:H7 adhesion to cells and reduced Stx cytotoxicity	Di al., 2017 [190]
	probiotics	*In vitro* (*E.coli* O104:H4 and O157:H7)	Inhibited STEC growth and Stx espresion	Mohsin *et al*.,2015 [193]
		In vitro (*E.coli* O104:H4 and O157:H7)	Reduced STEC growth and inhibited Stx release	Rund 2013 [194]
		In vitro (several STEC strains)	Reduced STEC growth and inhibited Stx release	Reissbrodt *et al*., 2009 [195]
Stem cells	Muse cells	*In vivo* (mice model)	Protected mice chalenged with STEC and prevented neuronal damage from Stx	Ozuru 2019 [198]
Proteasome inhibitor	Bortezomib	*In vitro* (THP1 and U937 cells)	Protected cells against Stx	Hattori *et al*., 2015 [201]
		In vivo (mice)	Protected mice challenged with Stx	
Inhibitor of Gb3 synthesis	C-9	*In vivo* (rat model)	Protected mice challenged with Stx	Silberstein *et al*., 2011 [202]
Immunoglobulin G depletion	Immunoglobulin G depletion	Case report	Improved in neurological and renal function	Flam *et al*., 2016 [203]
Lipids	Lysophospholipids	*In vitro* (HEp-2 cells)	Inhibited the binding of Stx to Gb3 and its toxicity	Aite *et al*., 2016 [205]
Retrograde transport inhibitor	Retro-1, Retro-2 & Retro-2^{cycl}	*In vitro* (He La cells)	Retro-1 and Retro-2 protected the cells against Stx	Stechmann *et al*., 2010 [206]
		In vivo (murine model)	Retro-2^{cycl} protected mice chalanged with STEC O104:H4	Secher *et al*., 2015 [208]
		In vitro (He La cells)	Retro-1 protected the cells against Stx	Abdelkafi *et al*., 2015 [207]
		In vivo (murine model)	Retro-2 protected mice chalanged with STEC O104:H4	Gupta *et al*., 2017 [209]

(Table 1) contd….

Drug Class	Drug	Type of Study	Effect	Refs.
	Tamoxifen	*In vitro* (He La cells)	Inhibited the trafficking and toxicity of Stx	Selyunin *et al*., 2019 [213]
		In vivo (mice)	protected mice from a lethal dose of Stx	
Metals	Bismuth	*In vitro* (*E. coli* O157:H7 & O104:H21)	Reduced the bacterial growth	Subils *et al*., 2014 [215]
		In vitro (*E. coli* O157:H7)	Reduced the bacterial growth an production of Stx	Pitz *et al*., 2015 [214]
	Zinc	*In vitro* (T84 cells)	Prevented the translocation of Stx into cel monolayers and inhibited SOS system expression	Crane *et al*., 2014 [216]
	Manganese	*In vitro* (He La cells)	Protected cells against Stx	Tewari *et al*., 2014 [217]

seems to be currently available to treat STEC-derived encephalopathy. In the last few years, several drugs have been experimentally employed to establish the pathogenesis of, to prevent or to treat CNS injury by STEC, with strategies relying on drug capability to inhibit either toxin entry into the cerebral parenchyma or its deleterious action once inside the CNS. Therefore, the therapeutic strategies that we postulate and describe in this review and which have been or may be used are: 1) inhibition of Stx production and release by STEC, 2) inhibition of Stx bloodstream transport, 3) inhibition of Stx entry into the CNS parenchyma, 4) blockade of deleterious Stx action in neural cells and 5) inhibition of immune system activation and CNS inflammation (Figs **2** and **3**). Table **1** describes a quick description of the drugs analyzed in this review.

4.1. Antibiotics used to Reduce STEC Encephalopathies

4.1.1. Polymyxin

Antibiotic administration is not recommended in the prodromal intestinal phase of human STEC infections, as it induces the phage lysogenic cycle with the consequent increase in expression and release of Stx2 [17]. However, strong evidence suggests that the antibiotic polymyxin B blocks the interaction of Stx with human neutrophils and impairs their capability to respond to Stx, inhibiting the release of chemokines and pro-inflammatory cytokines such as CXCL8, TNF-α and IL-1B [79]. This blockade is most likely to take place through the interaction of polymyxin B with the A chain of Stx, responsible for binding with human neutrophil TLR4 [50, 54, 79-84]). Polymyxin B appears to exert the same protective effect against LPS [85], inhibiting its interaction with TLR4 present in monocytes and platelets and preventing the activation of these cells and the upregulation of Gb3 produced by pro-inflammatory cytokines, as discussed above. In addition to polymyxin B, *in vitro* studies have shown that polymyxin E (colistin) is also efficient in inhibiting bacterial growth and Stx production by *E. coli* O157:H7 [86].

Polymyxin is a class of polypeptide antibiotics developed in the 1940s, which consists of amphipathic lipopeptide molecules derived from the fermentation products of bacteria *Paenibacillus polymyxa*. Both polymyxin B and colistin are bactericidal antibiotics whose mode of action remains controversial [79, 86-89]. It was suggested that they may act as an amphipathic detergent producing pore-like aggregates, which culminates in bacterial death, as well as the inhibition of cytokine release and LPS neutralizing effects [88, 89]. As they do not cause damage in bacterial DNA, the bacterial SOS response (response to DNA damage which promotes the transcription of Stx genes carried by the bacteriophage genome) is not elicited, and Stx genes are therefore not expressed before cell death [79, 86].

However, caution is necessary for the study of Stx inhibition by polymyxin B. As the antibiotic blocks the interaction between StxA and TLR4, it would be pointless to study it in a murine model, since murine PMN expresses Gb3 as well as TLR4 [82], in contrast with human PMN, which only expresses TLR4 [90]. In these conditions, StxB will be preferentially bound to Gb3 [82]. As a consequence, other animal models should be employed to establish the efficacy of polymyxin B and its derivatives to block the Stx effect [79]. Two major adverse effects in polymyxin treatment are nephrotoxicity and neuromuscular joint blockade. These effects, however, could be bypassed through oral administration, as polymyxin is only slightly absorbed and mostly eliminated by the gastrointestinal tract [86]. In sum, more research is needed on polymyxin beneficial effects.

4.1.2. Fosfomycin

Fosfomycin is a bactericidal broad-spectrum antibiotic first isolated in 1969 from cultures of *Streptomyces* spp. This antibiotic has a unique mechanism of action in which it reduces penicillin-binding proteins and inhibits the first step in peptidoglycan biosynthesis, leading to bacterial cell lysis and death. It reaches the bacterial cytoplasm using both the hexose monophosphate and the L-a-glycerophosphate transport system [91].

A study of 118 children with STEC infection between 1997 and 2013 [92] determined that the use of fosfomycin within the first 5 days of STEC infection restricted the development of HUS. Furthermore, another study of 292 children with *E. coli* O157 infection [93] reported that fosfomycin should be administered in a rather smaller time window, during the first 2 days of illness, in order to reduce the risk of HUS. However, a case report in 2017 [94] described the evolution of a 20-year-old woman whose digestive symptoms improved after fosfomycin treatment four days from the beginning of gastroenteritis with EHEC O26, who was then diagnosed with HUS a day after treatment. This patient be-

gan supportive care treatment with improvements in symptoms but developed acute encephalopathy five days later. Subsequent treatment with 1g/day methylprednisolone pulse therapy (mPSL) for three days later proved to be beneficial, as neurologic symptoms, hemolytic anemia, low platelet count and renal dysfunction all improved. The patient was finally discharged without any sequelae 23 days later.

4.1.3. Azithromycin

Azithromycin, a broad-spectrum macrolide antibiotic with bacteriostatic activity against many Gram-positive and Gram-negative bacteria, has also been reported as a possible therapeutic strategy to be employed due to its potential beneficial effect in the prevention of HUS and/or encephalopathy derived from infection with STEC [95]. In contrast to newer fluoroquinolones [96, 97], azithromycin has been able to inhibit the *in vitro* growth of STEC strains without the production of Stx. Another characteristic of this class of antibiotics is its immunomodulatory activities, such as the inhibition of neutrophil chemotaxis and oxidative burst and the release of pro-inflammatory cytokines from monocytes [98-101]. Azithromycin can also inhibit the Stx-induced production of inflammatory cytokines TNF-, IL-1 and IL-6 and prevent death in mice exposed to Stx or STEC [95].

4.2. Anti-inflammatory Drugs

4.2.1. Corticosteroid

Given the importance of the pro-inflammatory response produced by Stx and LPS, several authors have reported the use of anti-inflammatory steroids in clinical cases and in animal models of encephalopathy produced by Stx. Clinically, corticosteroid therapy has been used since the early 1960s [102, 103], although controversial results in therapy success have also been reported [104].

4.2.1.1. Betamethasone

Betamethasone is a synthetic, long-acting corticosteroid with high potency glucocorticoid (25 times more potent than cortisol) but minimal mineralocorticoid activity. Betamethasone pulse therapy has been found to reduce the mortality rate and improve the survival period of Stx2-toxemic rabbits with doses 8 times higher than those used in humans [105].

4.2.1.2. Methylprednisolone

Still, currently in use, methylprednisolone therapy has been extensively reported to exert neuroprotective effects and reverse CNS damage without sequelae [59, 60, 94, 106-110].

4.2.1.3 Dexamethasone

As corticosteroid is used in animal models, dexamethasone has proven to be a good neuroprotective candidate and is one of the most common corticosteroids used in medicine. It has a biological response thirty times more potent than endogenous cortisol and, in contrast to the latter, has virtually no mineralocorticoid effects [111]. Many of the anti-inflammatory effects of glucocorticoids are mediated by their inhibitory action on inflammatory cells, the generation of arachidonic acid-derived pro-inflammatory mediators and cytokine production [112]. Furthermore, dexamethasone blocks platelet aggregation [113] and increases the expression of occludin, a protein present in BBB endothelial tight junctions [111], which makes the BBB less permeable.

Dexamethasone has been shown to increase the survival of mice challenged with two lethal doses of Stx2 and, consequently, to protect neuronal populations, reduce astrocyte/microglial reactivity, myelin sheath damage and BBB permeability, and restore endothelial VEGF basal expression [56, 105, 114-116]. Overall, treatment with dexamethasone results in improved motor behavior.

4.2.2. Etanercept

This anti-inflammatory drug is a TNF-α inhibitor which blocks its interaction with cell surface TNF-α receptors. Etanercept is a dimeric fusion protein consisting of the extracellular ligand-binding portion of the human TNF-α receptor linked to the Fc portion of human IgG1 and is produced by recombinant DNA technology in a Chinese hamster ovary mammalian cell expression system [117, 118]. Etanercept has a binding affinity to TNF-α, which is up to 1000-fold higher than that of the natural TNF-α receptors [119]. Etanercept has been reported to protect animals against a lethal dose of Stx2 through a reduction in neuronal Stx2 uptake [116]. As etanercept promotes the clearance of TNF-α, it may be thought to prevent Gb3 neuronal up-regulation induced by TNF-α and other pro-inflammatory cytokines [58, 116].

However, the anti-inflammatory efficacy of etanercept is reduced in prolonged administration. Although the underlying mechanisms have not been described yet, a possible explanation lies in the development of anti-etanercept antibodies. This effect may be prevented through combined treatment with corticosteroids, as recommended in the case of psoriatic arthritis [120].

4.3. Vasoactive Drugs

4.3.1. Angiotensin 1-7

A recent article reported that angiotensin 1-7 succeeded in preventing neural damage following the intra-cerebroventricular treatment of Stx2 [121]. For many years since the development of the first oral inhibitor of the angiotensin-converting enzyme (ACE), responsible for the conversion of angiotensin I to angiotensin II, the renin-angiotensin system (RAS) has been the primary therapeutic target for the treatment of hypertension and related diseases. An alternative pathway for angiotensin metabolism and signaling is related to the action of the angiotensin-converting enzyme 2 (ACE2), which transforms angiotensin II into angiotensin 1-7 [122]. The latter exerts its effect through a unique G-protein-coupled receptor known as MAS. The activation of MAS produces anti-inflammatory, antioxidant, vasodilatory and angiogenic effects, which have proven beneficial in many animal models of CNS damage by stroke [123-127] and inflammation-related disease models including arthritis, hypertensive kidney disease, atherosclerosis, asthma and acute respiratory distress syndrome [128-132]. In this model, Stx2 produced neurodegeneration, demyelination and astro-

cyte damage, accompanied by edema, which angiotensin 1–7 was able to prevent in neurons and oligodendrocytes, but partially only in astrocytes. On the other hand, angiotensin 1-7 failed to prevent *in vitro* oligodendrocyte damage produced by Stx2, which suggests that its protective effects may be mediated by its neuronal receptor [133, 134], with a key role of cellular interaction.

4.3.2. Anisodamine

Another vasoactive drug employed in Stx-producing disease models, anisodamine (6-[s]hydroxyhyoscyamine), is an atropine derivative from *Scopaliatangutica* [135]. This drug is a non-specific cholinergic antagonist with respect to M1 and M2 receptors and appears to be less potent and toxic than atropine [136]. Anisodamine has vasodilating activity due to its relatively weak α1 adrenergic antagonism, and experimental evidence suggests antioxidant and superoxide scavenging activity as well [135]. Moreover, anisodamine presents antithrombotic activity, as it inhibits thromboxane synthesis [137]. Broad therapeutic uses have been proposed, including disorders related to the autonomic nervous system, migraine, gastric ulcers, gastrointestinal colic, acute glomerulonephritis, eclampsia, respiratory diseases, rheumatoid arthritis, snake bites and radiation damage, among others [135].

In Stx1-producing disease models, anisodamine has been able to increase the survival of Stx1-treated mice, a beneficial effect which may be due to the inhibition of proinflammatory cytokine TNF-α, IL-1β and IL-8 synthesis by human peripheral blood monocytes *via* a PGE2-dependent mechanism. Furthermore, anisodamine may also produce a beneficial effect by improving microvascular circulation [138].

4.4. Antibodies

4.4.1. Eculizumab

Several authors have reported hypocomplementemia in approximately one-third of children with HUS [139-146]. This clinical sign is strongly associated with severe episodes of HUS-derived encephalopathy and with poor prognosis [140, 147]. It is not clear why Stx promotes the complement system activation only in a fraction of affected children; however, children with hypocomplementemia are known to be significantly younger than those with normal blood complement levels [140].

Circulating Stx directly activates the complement system and also binds and neutralizes factor H, a soluble complement regulator essential for controlling the alternative pathway [148]. Therefore, treatment with a complement inhibitor may be beneficial to prevent STEC-derived encephalopathies and, consequently, death from this disease.

Accordingly, many reported cases of STEC-derived encephalopathies describe the therapeutic use of the C5 complement molecule inhibitor eculizumab [68, 140, 141, 148-152]. Eculizumab is a humanized chimeric monoclonal antibody comprising a human constant region and a murine complementarity determining region grafted onto human framework light and heavy chain variable regions. It is designed to bind one or two C5 molecules to inhibit their activation and thus block terminal complement activation [148, 153, 154].

Although the pathogenic mechanism in encephalopathy produced by Stx is not yet fully understood, evidence suggests direct damage of neural cells by the toxin [155], indirect cerebral damage by inflammation [56, 115, 116] and cerebral thrombotic microangiopathy [156]. Complement-mediated neurological impairment in STEC infections has been described by many authors and early treatment with eculizumab has proven more efficient than plasmapheresis in improving neurological status [69]. In contrast, late eculizumab treatment following unsuccessful treatment with plasmapheresis has shown no benefits [157].

4.4.2. Passive and Active Immune Therapy

A logical strategy to protect the CNS against the detrimental action of Stx is the employment of passive immune therapy with anti-Stx antibodies or active immune therapy to neutralize the toxin long before brain damage occurs. In this context, pre-clinical studies using chimeric murine-human monoclonal antibodies have been developed since the late 1980s [158] and are, until now, the most effective treatment so far. *In vitro* and *in vivo* models [159-164] using monoclonal antibodies have demonstrated success in neutralizing the toxic and lethal effects of Stx, respectively. Recently, healthy adult volunteers have received treatment with anti-Stx antibodies corresponding to phase 1 safety and pharmacokinetic trials [159, 165-167]. These studies showed that all these antibodies were well tolerated, which makes them good candidates to be employed in the next phase of clinical trials.

Neutralizing antibodies targeting StxB to prevent the binding of Stx to Gb3 constitute the theoretical basis of active immunization against Stx. However, as it turns out to be StxB a poor immunogen researchers developed a vaccine with an engineered chimeric molecule consisting of StxB bound to *Brucellaspp*'s enzyme lumazine synthase [168, 169]. This new immunogen molecule has demonstrated a strong capacity to induce long-lasting humoral immune response with a high neutralizing capacity for Stx2 in a murine model of systemic delivery of Stx2 or even oral challenge of EHEC. This novel immunogen represents a promising candidate for vaccine or antibody development with preventive or therapeutic ends. Furthermore, female mice immunized before mating with the chimeric *Brucella* lumazine synthase-StxB developed a strong humoral response, while their offspring acquired a very similar titer of the antibody through transplacental and breastfeeding. Moreover, pups were totally protected against systemic injection of a lethal dose of Stx up to 3 months postpartum and against EHEC infection at weaning [168].

4.5. Polyphenols

Polyphenols are phytochemicals primarily found in fruits, vegetables, cereals and beverages, which are generally involved in defense against ultraviolet radiation or aggression by pathogens. More than 3 mg of polyphenols can be found per gram in fresh weight fruits like grapes, apples, pears, cherries and berries [170]. Epidemiological and associated

meta-analysis studies strongly suggest that long-term consumption of diets rich in plant polyphenols offers protection against cancer, cardiovascular disease, diabetes, osteoporosis, neurodegenerative diseases and infections [171-173]. The beneficial action of polyphenols may be due to the presence of an antioxidant phenolic group which can accept an electron to form a relatively stable phenosyl radical, thus increasing plasma antioxidant capacity [174]. Non-identified polyphenols from grape seed and pomace extracts were reported as molecules with potential inhibitory power of the toxic effect of Stx, as observed in Vero cells [175].

4.5.1. Baicalin

Baicalin (5, 6-dihydroxy-7-O-glucuronide flavone) is a polyphenol from *Scutellaria baicalens* employed in traditional Chinese medicine for the treatment of lung and liver diseases, diarrhea, dysentery, high blood pressure, bleeding, insomnia and inflammation [176]. Baicalin presents various pharmacological properties, including anti-oxidative, antiviral, anti-inflammatory, anti-HIV and antineoplastic activity [177].

Baicalin has been able to protect HeLa [178, 179] and Vero cells [180] against Stx2, either after pre-treatment with Baicalin or even upon pre-incubation with Stx2. In turn, mice were challenged either with a lethal dose of Stx [178] or a lethal charge of *E. coli* O157:H7 [179], both to be later treated with oral baicalin, showing 70% and 80% protection, respectively. Furthermore, baicalin reduced the systemic production of IL-1, IL-4, IL-6, TNF-α and INF-γ in both models. Although baicalin showed no effects on the production or secretion of Stx, docking models revealed that the inhibitory activity of baicalin against Stx might be due to the formation of a binding structure inside the pocket of the StxB pentamers [180].

4.5.2. Catechins: Gallocatechin Gallate (GCg) and Epigallocatechin Gallate (EGCg)

Catechins are natural polyphenolic compounds found mainly in tea leaf (*Camellia sinensis*) and other vegetables [181-184]. These compounds have many valuable properties such as anti-bacterial, anti-viral, anti-oxidative and anti-tumor activity [185-188].

From six different catechin derivatives contained in green tea extracts, GCg and EGCg were found to inhibit bacterial growth. Furthermore, GCg and EGCg that suppressed the release of Stx from STEC failed to block the production of Stx, as they act by inhibiting the leakage of molecules from periplasm [181].

4.6. Pharmabiotics

Pharmabiotic is a general term denoting the therapeutic use of commensal microbiota, which includes the use of prebiotics and live probiotic microorganisms [189].

4.6.1. Prebiotics

Prebiotics are selectively fermented non-digestible food ingredients which induce specific changes in the composition and/or activity of the gastrointestinal microbiota and thus offer benefits to host health [190]. Typical prebiotics, such as fructo- or galacto-oligosaccharides or lactulose, are selectively fermented and thus increased by beneficial bacteria in the colon [191]. Pectic oligosaccharides are derived from pectin, a polysaccharide found in high amounts in citrus fruits, which presents a partially methylesterified homogalacturonan backbone [192]. When pectin is consumed, it reaches the colon and becomes fermented by microbiota, hence yielding oligosaccharides and smaller metabolites [192]. *In vitro* studies have demonstrated the protective role of pectin and pectic-oligosaccharides against Stx lethality in human colonic cell line HT29 [193]. Furthermore, pectic-oligosaccharides showed anti-adhesive properties, inhibiting the binding of *E. coli* O157:H7 to human HT29 cells [194].

4.6.2. Probiotics

Probiotics are live non-pathogenic microorganisms (*Saccharomyces boulardii* yeast or lactic acid bacteria, such as *Lactobacillus* and *Bifidobacterium* species), which, when administered in adequate amounts, provide health benefits to the host [189, 195, 196]. Probiotics exert their beneficial effects through various mechanisms, including lowering intestinal pH, decreasing colonization and invasion by pathogenic organisms and beneficially modifying the host immune response. Probiotics have been commonly targeted at illnesses associated with the gastrointestinal tract, mainly due to their ability to restore gut microbiota [196].

Escherichia coli Nissle 1917 (EcN) is a probiotic strain which has displayed an inhibitory effect on toxin growth and gene expression, Stx release and cytotoxicity in STEC serotypes O104:H4 and O157:H7 [197]. Furthermore, adhesion of STEC strains to Caco-2 cells and mucin-producing LS 174T cells has been significantly reduced upon co-culture with EcN [198]. On the other hand, routine quality control testing of STEC rendered Stx detection in only about 39% of all samples tested positive for STEC strains [199]. A particular *E. coli* strain isolated from stool samples of STEC-infected Stx-negative patients, the non-probiotic *E. coli* 1307 proved to possess probiotic like-properties similar to those of EcN, as it reduced bacterial growth and inhibited Stx release when co-cultured with STEC strains [199].

4.7. Stem Cells

4.7.1. Multilineage-differentiating Stress-enduring (Muse) Cells

Muse cells are endogenous non-tumorigenic stem cells with the ability to sense, migrate to and populate the site of tissue damage, therefore replenishing it with new functional cells by spontaneous differentiation and inducing functional and structural repair. They are also stress-tolerant cells which perform anti-fibrotic, anti-inflammatory and anti-apoptotic functions. Due to their immunomodulatory effect, allograft and xenograft Muse cells may escape from host immunologic attack and thus efficiently settle in the damaged site. Muse cells can be intravenously administrated to patients after collection and expansion from tissue sources [200]. Moreover, Muse cells have been applied to clinical trials for acute myocardial infarction, stroke and spinal cord injury by intravenous injection of donor-derived cells without HLA-matching or immunosuppressant treatment [201]. Muse cells

have also shown a beneficial effect on STEC-associated acute encephalopathy, as they protected 100% of mice against lethal treatment with STEC O111. Immunohistochemical analysis showed a decrease in astrocyte reactivity and caspase-3 inhibition, accompanied by a neuron-like morphology of the human Muse cells detected through an anti-Cox4 antibody in the mouse brain parenchyma. These findings suggest that Muse cells produced a beneficial effect by crossing the BBB. Furthermore, a single injection of as few as $5x10^4$ Muse cells 48h after O111 infection proved to prevent neuronal damage by Stx [202].

Among the compounds produced by Muse cells, the granulocyte-colony-stimulating factor (G-CSF) has been unequivocally identified as a neuroprotectant, as the suppression of G-CSF by iARN reduced the beneficial effects of these cells. G-CSF has also been observed to maintain aquaporin type 4 integrity and induce vascular endothelial growth factor, which improved neurologic functions by reducing brain edema, BBB permeability, neuronal death and apoptosis [203]. G-CSF also stimulated the proliferation of neuronal/glial progenitor cells, producing an improvement in neurocognitive function, and succeeded in repairing cerebral white matter damage by irradiation [204].

4.8. Proteasome Inhibitor

It has been reported that the clinically approved proteasome inhibitor bortezomib manages to inhibit Stx1-induced apoptosis *in vitro* through the mitochondrial pathway of caspases 9-3. It was observed *in vitro* that the levels of apoptosis inhibitory proteins decreased due to proteasomal degradation, followed by caspase activation and apoptosis progression. In this context, proteasome inhibitors succeeded in preventing the Stx-induced reduction in anti-apoptotic proteins Apollon, XIAP, c-IAP1, FLIP and Mcl-1 and, consequently, the progression of apoptosis. Furthermore, *in vivo* bortezomib administration prolonged the survival of mice intoxicated with Stx. Therefore, even if high 2mg/kg doses of bortezomib in the present study proved to be toxic and its potential clinical use should be handled with caution, inhibition of the proteasome may be beneficial in the treatment of patients affected by Stx intoxication [205].

4.9. Gb3 Synthesis Inhibitor

Another strategy to prevent the deleterious action of Stx is to reduce the expression of the Stx Gb3-receptor using C-9 [(1R, 2R)-nonanoic acid [2-(2′, 3′-dihydro-benzo (1–4) dioxin-6′-yl)-2-hydroxy-1-pyrrolidin-1-ylmethyl-ethyl]-amide-L-tartaric acid salt], a competitive inhibitor of the glucosylceramide synthase, responsible for rate-limiting the first step in the biosynthesis of Gb3 and globosides in general [206]. The use of this drug, administered 2 days before Stx2 systemic delivery, managed to reduce the mortality rate in rats by 50%. However, the fact that C-9 does not cross the BBB may explain its failure in attaining total survival. Alternatively, as C-9 inhibits the expression of the whole globoside series and knowledge currently available on Gb3 and other globosides biological functions is still limited, this drug may be thought to interfere with physiological functions, which calls for more preclinical studies with this drug in the treatment of the systemic effects of STEC infection.

4.10. Immunoglobulin G (IgG) Depletion

Another type of treatment employs IgG depletion by immunoadsorption, which has succeeded in rapidly improving neurological and renal functions. However, as the specificity of the antibody toward CNS antigens could not be identified, authors hypothesize that the effect of IgG depletion could be due to the elimination of autoantibodies or harmful immune complexes with Stx. Therefore, it has been postulated that late neurological involvement in STEC-HUS may be mediated by immune factors [207].

4.11. Lysophospholipids

In vitro studies have shown that the conformation quality of the lipid bilayer of the plasma membrane could determine the binding of Stx to the Gb3 receptor [208]. In this regard, incubation of lysophospholipids such as lysophosphatidylinositol, which has a large polar head with an extensive and saturated fatty acyl chain, has a conical structure that inhibits the binding of Stx or Stx2 to Gb3 and, hence, toxicity in HEp-2 cells. In contrast, this does not happen with lysophospholipids having a small polar head and non-saturated shorter fatty acyl chains, which give a cylindrical structure. Therefore, lysophospholipids such as lysophosphatidylinositol change the physicochemical properties of the plasma membrane, which leads to alterations in the conformation and/or distribution of the Gb3 receptor [209].

4.12. Retrograde Transport Inhibitor

4.12.1. Retro-1, Retro-2 & Retro-2^{cycl}

Another strategy to neutralize the cytotoxic effect of Stx is inhibiting the retrograde transit of this toxin inside the cells. Three components have been found to specifically block the early endosome-to-trans Golgi network toxin transport step without affecting endogenous retrograde cargo proteins, other intracellular trafficking pathways, or, more generally, organelle integrity. It was observed that Retro-1 and Retro-2 protected HeLa cells from the lethal effect of Stx [210, 211]. Moreover, Retro-2 and Retro-2^{cycl} protected mice against a challenge with a lethal oral charge of STEC O104:H4 [212, 213].

4.12.2. Tamoxifen (TAM)

TAM is an antiestrogenic drug widely used for the treatment of estrogen receptor-α-positive breast cancer [214]. TAM inhibits the trafficking and toxicity of Stx in HeLa cells, which lack estrogen receptors but are sensitive to Stx [215, 216]. This protective effect is independent of estrogen receptors but rather more related to TAM weak base properties, which increase endolysosomal pH. This property results from the chemical structure of TAM, characterized by a tertiary amine group. As a result, TAM has been shown to protect mice from a lethal dose of Stx [217].

4.13. Divalent and Trivalent Metals

The antibacterial effects of certain trivalent/divalent metals have been demonstrated in recent years. Bismuth salts or colloidal bismuth hydroxide gel have been observed to reduce the bacterial growth of *E. coli* O157:H7 and *E coli* O104:H21 *in vitro* within 48 hours, as evidenced by the degradation of

the cell wall, inhibition of plasma membrane function and impediment of protein and ATP synthesis [218, 219].

It has been reported that zinc offered protection against STEC infection by reducing the expression of the SOS system in the presence of hydrogen peroxide, xanthine oxidase or antibiotic ciprofloxacin in the gastrointestinal tract. Zinc also had protective effects on enterocytes, by restoring the impermeability of tight junctions, on the one hand, and preventing the translocation of Stx into monolayers, on the other [220].

Finally, manganese has been used to degrade cycling transmembrane protein GPP130, which Stx uses as an intracellular traffic receptor. The role of manganese lies in its union with GPP130, which blocks toxin movement to the Golgi to be led to lysosomes, where it is degraded and purified from infected cells. Manganese treatment could then be accessible, given its low cost and availability [221].

CONCLUSION

At present, there is no consensus high-efficiency treatment for STEC infections and only supportive treatment is employed for STEC-infected patients. Once the patient is diagnosed, hospitalization and volume expansion therapy are required to monitor the progression of the disease and maintain the level of hydration, respectively [159]. In this review, we intended to provide a wide range of therapeutic strategies aimed, on the one hand, to eliminate the bacteria from the gut without producing and releasing Stx and, on the other hand, to block the action and the effect of Stx systemically and locally in the CNS.

An important question to be solved is when to start an effective treatment. There are only a few clinical studies reporting a therapeutic time window for effective pharmacological treatment (fosfomycin within the first 2 to 5 days of STEC infection [92, 93]). Furthermore, the administration of Gb3 analogs orally provided to positively diagnosed diarrhea-HUS-patients has been tested as a treatment specifically aimed to neutralize the toxin at the intestinal level [222]. The study showed that patients who received Gb3 analogs had similar clinical evolution to those who received the placebo. This evidence suggests that the time window for therapeutic approaches aiming at preventing toxin release by bacteria and/or toxin arrival at the bloodstream is shorter than that required for drugs of systemic action. Therefore, fast diagnosis of STEC infection, as well as the establishment of early biomarkers of CNS damage, may be crucial to a rapid pharmacological approach. Early treatment by early diagnosis may lead to a better prognosis and a reduction in death or sequelae.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

This work was supported by Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) (PICT-2016-1175) and Universidad de Buenos Aires (UBACyT) (20020160100135BA), Argentina.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We were grateful to María Marta Rancez for her special dedication and technical assistance.

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