Therapeutic Options of Enterohemorrhagic *Escherichia coli* Infections

Subjects: Gastroenterology & Hepatology

Contributor: Johanna Detzner, Gottfried Pohlentz, Johannes Müthing

Enterohemorrhagic *Escherichia coli* (EHEC) are the human pathogenic subset of Shiga toxin (Stx)-producing *E. coli* (STEC). EHEC are responsible for severe colon infections associated with life-threatening extraintestinal complications such as the hemolytic-uremic syndrome (HUS) and neurological disturbances. Endothelial cells in various human organs are renowned targets of Stx, whereas the role of epithelial cells of colon and kidneys in the infection process has been and is still a matter of debate.

Keywords: EHEC ; glycolipids ; lipid rafts ; STEC

1. Application of Antibiotics or Not That's the Question

The administration of antibiotics in EHEC infections was and remains controversial because of concerns about triggering HUS by increasing Stx production [1][2][3]. Stxs are encoded by genes located on genomes of lambdoid prophages and certain antibiotics stimulate their induction leading to enhanced production of Stxs [4]. Although numerous studies have reported that antibiotics enhance the severity of disease symptoms and increase the risk of progression to HUS development, further corroborated by in vitro antibiotic studies using certain EHEC strains, others have reported that antibiotics do not have any effect or can even reduce the rate of HUS development in EHEC infections [5][6][7][8][9][10][11][12]. The current data situation leads to the conclusion that the infecting EHEC strain, the type of antibiotic, and the timing of its application appear to significantly affect the development of HUS in EHEC-infected patients [1].

2. Development of Non-Antibiotic Therapeutics

In recent years, a variety of alternative treatment approaches and therapeutic interventions has been developed and evaluated in vitro, in animal models and clinical trials for preventing EHEC-associated HUS [13][14]. The majority of possible non-antibiotic therapeutics has been or is in the developmental stage aimed to neutralize Stx, to prevent toxin adhesion, to block receptor biosynthesis, and to interfere trafficking, processing, and activity of the toxin within the cell [9][15][16][17][18][19]. Since Stx induces the secretion of inflammatory cytokines and chemokines from susceptible cells that contribute to the pathogenesis of HUS, these compounds are useful indicators of disease activity as well as predictors of disease progression and candidates for an anti-inflammation therapy as an additional treatment regimen for severe *E. coli*-associated HUS [20].

2.1. Inhibitors of Glycosphingolipid Biosynthesis and Stx-Neutralizing Glycoconjugates

Ceramide is the hydrophobic backbone of all complex amphipathic glycosphingolipids (GSLs). Its initial glycosylation forming glucosylceramide (GlcCer) is the first committed and rate-limiting step in the biosynthesis of GSLs with GlcCer core leading to the various GSL-families including the globo-series [21]. A number of ceramide analogs such as classical D-PDMP and many others has been scrutinized in the past as potential inhibitors of GlcCer synthase mainly developed for the treatment of human lipid storage diseases named as substrate reduction therapy [22][23][24][25][26]. The capability of traditional and novel GlcCer synthase inhibitors to reduce the cellular level of the Stx receptor Gb3Cer in various cell types including human epithelial and endothelial cells [27][28] and to prevent the cytotoxic action toward this way Gb3Cer-truncated target cells has expedited an additional focus on the Stx receptor Gb3Cer as therapeutic target in Stx-mediated HUS. An example of a newly developed GlcCer-synthase inhibitor is the ceramide analog Eliglustat [26], also primarily developed as an alternative approach to the enzyme replacement therapy of patients suffering from GSL storage diseases [29], effectively protects human renal tubular epithelial cells from Stx-caused cellular damage due to reducing the cellular Gb3Cer levels suggesting its potential as Stx protector [30][31]. The prevention of Gb3Cer-synthesis and neutralization of Stx-mediated cytotoxic action by the ceramide analog C-9, shown for primary human renal epithelial cells in vitro and an in vivo animal HUS model in rats offer a further option for treatment of EHEC-HUS [32][33].

Aligned to the opposite site of an amphipathic GSL from the lipid anchor, modifications of the hydrophilic glycan represent a further approach to impede or prevent Stx binding. Such metabolic modification can easily be done and has been reported for feeding of in vitro propagated cells with 2-deoxy-D-glucose or 2-fluoro-2-deoxy-D-glucose revealing protective effects of both compounds against Stx [34][35]. 2-deoxy-D-glucose becomes incorporated into the carbohydrate moiety of GSLs and protects cells against Stxs [34], while 2-fluoro-2-deoxy-D-glucose inhibits GlcCer biosynthesis thereby reducing the cellular levels of GSLs as shown for various cell types including human brain microvascular endothelial cells. This glucose-modification is much more efficient in protecting cells against Stx when compared to 2-deoxy-D-glucose [35]. Furthermore, the clinically approved glucose-derivative Miglustat has been shown being effective in human endothelial and epithelial cells to decrease the level of Stx receptor Gb3Cer suggesting its application as a feasible strategy to protect kidney tissues from Stx-mediated kidney injury [36]. Collectively, the enumerated ceramide analogs and glucose-derivatives suggest potential clinical applications for Stx-caused diseases.

Since protein toxins of enterotoxic bacteria have proven to be attractive targets for drug development [18][37], numerous therapeutic glycoconjugates based on Stx-specific analogs of the glycan receptor Gb3 have been developed [13][38]. Synsorb Pk [39], Starfish [40], Daisy [41], SUPER TWIGS [42][43], polymeric acrylamide-Gb3 conjugates [44], Gb3 (glycan) encapsulated gold nanoparticles [45][46], neoglycolipid-spiked glycovesicles [47][48] or engineered probiotics expressing Gb3 analogs on their surface [49] are examples of glycoconstructs, developed for neutralization of Stxs as described more precisely in a nice and highly recommended recent review [13]. However, although effective in vitro, potential Stx-binding neutralizers have failed in vivo showing no benefit in clinical trials and none of them has received clinical approval to date [8][50].

2.2. Monoclonal Antibodies

Despite a tremendous increase of knowledge has been gained with regard to the generation of neutralizing humanized (chimeric) or human monoclonal anti-Stx antibodies to combat Stx-mediated diseases [51][52][53], so far no monoclonal antibody against Stx1(a) or Stx2(a) has received clinical approval [13][54]. The broadly administered anti-C5 monoclonal antibody Eculizumab during the 2011 outbreak of an O104:H4 EHEC strain in Germany gave an equally good outcome of treated versus untreated patients and pointed to an advantageous use, at least for severe cases [55]. This anti-C5 complement blocker has obviously made the difference between favorable or detrimental outcome [55][56]. The administration of Eculizumab in EHEC-associated HUS with neurological involvement indicated that early use of Eculizumab appeared to improve neurological outcome, whereas late treatment seemed to show less benefit suggesting advantage of prophylactic Eculizumab therapy before development of neurological symptoms [57]. Thus, treatment of EHEC-HUS patients with Eculizumab has shown positive clinical improvement and proven effective in some cases [50][58][59].

2.3. Further Alternative Therapeutic Concepts

Among further alternative therapeutic strategies, a promising approach is the use of probiotic microorganisms showing antagonistic effects on EHEC strains of various serotypes [8][60][61][62]. Suitable vaccine candidates against EHEC infections are polysaccharide conjugates such as constructs built up from *E. coli* O157 or *E. coli* O145 polysaccharides linked to bacterial carrier proteins offering high prospects for effective preventive treatment for future clinical studies [63][64]. Phage therapy using specific phages against *E. coli* O157:H7 has to be taken into consideration as well. More than 60 specific phages are known so far and in vitro experiments have been successful in elimination or reduction of *E. coli* O157:H7 numbers, but in vivo experiments have not been as promising [65]. The proof or principle of the novel antibiotic-peptide wrwycr has been reported effective in killing of EHEC in synergistic combination with antibiotic treatment without enhancing release of Stxs. This strategy offers a potential new candidate for a preventive antimicrobial for EHEC infections [66][67]. The retrograde transport of internalized Stx directly from early endosomes to the Golgi apparatus is an essential step to bypass degradation in the late endosomes and lysosomes, which then continues to the endoplasmic reticulum before translocation of the enzymatically active moiety to the ribosomal target in the cytosol [19][28][68][69][70][71][72]. This renders the crucial retrograde transportation route an ideal attack point for small molecule inhibitors of toxin trafficking as possible therapeutics acting at the endosome/Golgi interface [73][74]. Substances that interfere with intracellular trafficking inhibiting the transport of Stx have been summed in recent reviews [13][15] and will not be discussed further at this point.

2.4. Current Situation

A comprehensive study that summarized the results of clinical trials for preventing EHEC-associated HUS including antibiotics, the Stx inhibitor Synsorb Pk, and a monoclonal antibody against Stx (Urtoxazumab) revealed no firm conclusions about the efficacy of these interventions given the small number of included studies and their small sample

sizes [14]. Collectively, despite significant advances in understanding the molecular mechanisms of Stx being imperative for the design of appropriate drugs or adjunctive therapeutics, a rationally designed drug that targets Stx has yet not reached the market [13][14][50].

References

1. Kakoullis, L.; Papachristodoulou, E.; Chra, P.; Panos, G. Shiga toxin-induced haemolytic uraemic syndrome and the rol e of antibiotics: A global overview. J. Infect. 2019, 79, 75–94.
2. Biernbaum, E.N.; Kudva, I.T. AB5 enterotoxin-mediated pathogenesis: Perspectives gleaned from Shiga toxins. Toxins 2022, 14, 62.
3. Tarr, P.I.; Freedman, S.B. Why antibiotics should not be used to treat Shiga toxin-producing Escherichia coli infections. Curr. Opin. Gastroenterol. 2022, 38, 30–38.
4. Loś, J.M.; Loś, M.; Węgrzyn, G. Bacteriophages carrying Shiga toxin genes: Genomic variations, detection and potenti al treatment of pathogenic bacteria. Future Microbiol. 2011, 6, 909–924.
5. Ochoa, T.J.; Chen, J.; Walker, C.M.; Gonzales, E.; Cleary, T.G. Rifaximin does not induce toxin production or phage-me diated lysis of Shiga toxin-producing Escherichia coli. Antimicrob. Agents Chemother. 2007, 51, 2837–2841.
6. Bielaszewska, M.; Idelevich, E.A.; Zhang, W.; Bauwens, A.; Schaumburg, F.; Mellmann, A.; Peters, G.; Karch, K. Effect s of antibiotics on Shiga toxin 2 production and bacteriophage induction by epidemic Escherichia coli O104:H4 strain. A ntimicrob. Agents Chemother. 2012, 56, 3277–3282.
7. Corogeanu, D.; Willmes, R.; Wolke, M.; Plum, G.; Utermöhlen, O.; Krönke, M. Therapeutic concentrations of antibiotics inhibit Shiga toxin release from enterohemorrhagic E. coli O104:H4 from the 2011 German outbreak. BMC Microbiol. 2 012, 12, 160.
8. Thomas, D.E.; Elliott, E.J. Interventions for preventing diarrhea-associated hemolytic uremic syndrome: Systematic revi ew. BMC Public Health 2013, 13, 799.
9. Melton-Celsa, A.R.; O'Brien, A.D. New therapeutic developments against Shiga toxin-producing Escherichia coli. Micro biol. Spectr. 2014, 2.
10. Tarr, G.A.M.; Oltean, H.N.; Phipps, A.I.; Rabinowitz, P.; Tarr, P.I. Strength of the association between antibiotic use and hemolytic uremic syndrome following Escherichia coli O157H7 infection varies with case definition. Int. J. Med. Microbi ol. 2018, 308, 921–926.
11. Mühlen, S.; Ramming, I.; Pils, M.C.; Koeppel, M.; Glaser, J.; Leong, J.; Flieger, A.; Stecher, B.; Dersch, P. Identification of antibiotics that diminish disease in a murine model of enterohemorrhagic Escherichia coli infection. Antimicrob. Agent s Chemother. 2020, 64, e02159-19.
12. Ramstad, S.N.; Taxt, A.M.; Naseer, U.; Wasteson, Y.; Bjørnholt, J.V.; Brandal, L.T. Effects of antimicrobials on Shiga tox in production in high-virulent Shiga toxin-producing Escherichia coli. Microb. Pathog. 2021, 152, 104636.
13. Mühlen, S.; Dersch, P. Treatment strategies for infections with Shiga toxin-producing Escherichia coli. Front. Cell. Infec t. Microbiol. 2020, 10, 169.
14. Imdad, A.; Mackoff, S.P.; Urciuoli, D.; Syed, T.; Tanner-Smith, E.E.; Huang, D.; Gomez-Duarte, O.G. Interventions for pr eventing diarrhoea-associated haemolytic uraemic syndrome. Cochrane Database Syst. Rev. 2021, 7, CD012997.
15. Kavaliauskiene, S.; Lingelem, A.B.D.; Skotland, T.; Sandvig, K. Protection against Shiga toxins. Toxins 2017, 9, 44.
16. Bitzan, M. Treatment options for HUS secondary to Escherichia coli O157:H7. Kidney Int. 2009, 75, S62–S66.
17. Nishikawa, K. Recent progress of Shiga toxin neutralizer for treatment of infections by Shiga toxin-producing Escherichi a coli. Arch. Immunol. Ther. Exp. 2011, 59, 239–247.
18. Hall, G.; Kurosawa, S.; Stearns-Kurosawa, D.J. Shiga toxin therapeutics: Beyond neutralization. Toxins 2017, 9, 291.
19. Robert, A.; Wiels, J. Shiga toxins as antitumor tools. Toxins 2021, 13, 690.
20. Shimizu, M. Pathogenic functions and diagnostic utility of cytokines/chemokines in EHEC-HUS. Pediatr. Int. 2020, 62, 3 08–315.
21. Sandhoff, K.; Kolter, T. Biosynthesis and degradation of mammalian glycosphingolipids. Philos. Trans. R. Soc. Lond. B Biol. Sci. 2003, 358, 847–861.
22. Abe, A.; Wild, S.R.; Lee, W.L.; Shayman, J.A. Agents for the treatment of glycosphingolipid storage disorders. Curr. Dru g Metab. 2001, 2, 331–338.

23. Jeyakumar, M.; Butters, T.D.; Dwek, R.A.; Platt, F.M. Glycosphingolipid lysosomal storage diseases: Therapy and patho genesis. Neuropathol. Appl. Neurobiol. 2002, 28, 343–357.

24. Aerts, J.M.F.G.; Hollak, C.E.M.; Boot, R.G.; Groener, J.E.M.; Maas, M. Substrate reduction therapy of glycosphingolipid storage disorders. J. Inherit. Metab. Dis. 2006, 29, 449–456.

25. Larsen, S.D.; Wilson, M.W.; Abe, A.; Shu, L.; George, C.H.; Kirchhoff, P.; Showalter, H.D.H.; Xiang, J.; Keep, R.F.; Shay man, J.A. Property-based design of glucosylceramide synthase inhibitor that reduces glucosylceramide in the brain. J. Lipid Res. 2012, 53, 282–291.

26. Shayman, J.A. Eliglustat tartrate, a prototypic glucosylceramide synthase inhibitor. Exp. Rev. Endocrinol. Metab. 2013, 8, 491–504.

27. Legros, N.; Pohlentz, G.; Runde, J.; Dusny, S.; Humpf, H.U.; Karch, H.; Müthing, J. Colocalization of receptors for Shig a toxins with lipid rafts in primary human renal glomerular endothelial cells and influence of D-PDMP on synthesis and distribution of glycosphingolipid receptors. Glycobiology 2017, 27, 947–965.

28. Raa, H.; Grimmer, S.; Schwudke, D.; Bergan, J.; Wälchli, S.; Skotland, T.; Shevchenko, A.; Sandvig, K. Glycosphingolip id requirements for endosome-to-Golgi transport of Shiga toxin. Traffic 2009, 10, 868–882.

29. Cox, T.M. Eliglustat tartrate, an orally active glucocerebroside synthase inhibitor for the potential treatment of Gaucher disease and other lysosomal storage diseases. J. Curr. Opin. Investig. Drugs 2010, 11, 1169–1181.

30. Sánchez, D.S.; Fischer Sigel, L.K.; Balestracci, A.; Ibarra, C.; Amaral, M.M.; Silberstein, C. Eliglustat prevents Shiga to xin 2 cytotoxic effects in human renal tubular epithelial cells. Pediatr. Res. 2022, 91, 1121–1129.

31. Feitz, W.J.C.; Bouwmeester, R.; Van der Velden, T.J.A.M.; Goorden, S.; Licht, C.; Van den Heuvel, L.P.J.W.; Van de Ka r, N.C.A.J. The Shiga toxin receptor globotriaosylceramide as therapeutic target in Shiga toxin E. coli mediated HUS. M icroorganisms 2021, 9, 2157.

32. Silberstein, C.; Copeland, D.P.; Chiang, W.L.; Repetto, H.A.; Ibarra, C. A glucosylceramide synthase inhibitor prevents t he cytotoxic effects of Shiga toxin-2 on human renal tubular epithelial cells. J. Epithel. Biol. Pharmacol. 2008, 1, 71–75.

33. Silberstein, M.; Lucero, M.S.; Zotta, E.; Copeland, D.P.; Lingyun, L.; Repetto, H.A.; Iberra, C. A glucosylceramide synth ase inhibitor protects rats against the cytotoxic effects of Shiga toxin 2. Pediatr. Res. 2011, 69, 390–394.

34. Kavaliauskiene, S.; Skotland, T.; Sylvänne, T.; Simolin, H.; Klokk, T.I.; Torgersen, M.L.; Lingelem, A.B.D.; Simm, R.; Ekr oos, K.; Sandvig, K. Novel actions of 2-deoxy-D-glucose: Protection against Shiga toxins and changes in cellular lipids. Biochem. J. 2015, 470, 23–37.

35. Kavaliauskiene, S.; Torgersen, M.L.; Lingelem, A.B.D.; Klokk, T.I.; Lintonen, T.; Simolin, H.; Ekroos, K.; Skotland, T.; Sa ndvig, K. Cellular effects of fluorodeoxyglucose: Global changes in the lipidome and alteration in intracellular transport. Oncotarget 2016, 7, 79885–79900.

36. Girard, M.C.; Sacerdoti, F.; Rivera, F.P.; Repetto, H.A.; Ibarra, C.; Amaral, M.M. Prevention of renal damage caused by Shiga toxin type 2: Action of Miglustat on human endothelial and epithelial cells. Toxicon 2015, 105, 27–33.

37. Ivarsson, M.E.; Leroux, J.C.; Castagner, B. Targeting bacterial toxins. Angew. Chem. Int. Ed. Engl. 2012, 51, 4024–404 5.

38. MacConnachie, A.A.; Todd, W.T.A. Potential therapeutic agents for the prevention and treatment of haemolytic uraemic syndrome in Shiga toxin producing Escherichia coli infections. Curr. Opin. Infect. Dis. 2004, 17, 479–482.

39. Trachtman, H.; Cnaan, A.; Christen, E.; Gibbs, K.; Zhao, S.; Acheson, D.W.K.; Weiss, R.; Kaskel, F.J.; Spitzer, A.; Hirsc hman, G.H.; et al. Effect of an oral Shiga toxin-binding agent on diarrhea-associated hemolytric uremic syndrome in chil dren: A randomized controlled trial. JAMA 2003, 290, 1337–1344.

40. Kitov, P.I.; Sadowska, J.M.; Mulvey, G.; Armstrong, G.D.; Ling, H.; Pannu, N.S.; Read, R.J.; Bundle, D.R. Shiga-like toxi ns are neutralized by tailored multivalent carbohydrate ligands. Nature 2000, 403, 669–672.

41. Mulvey, G.L.; Marcato, P.; Kitov, P.I.; Sadowska, J.; Bundle, D.R.; Armstrong, G.D. Assessment in mice of the therapeut ic potential of tailored, multivalent Shiga toxin carbohydrate ligands. J. Infect. Dis. 2003, 187, 640–649.

42. Nishikawa, K.; Matsuoka, K.; Kita, E.; Okabe, N.; Mizuguchi, M.; Hino, K.; Miyazawa, S.; Yamasaki, C.; Aoki, J.; Takashi ma, S.; et al. A therapeutic agent with oriented carbohydrates for treatment of infections by Shiga toxin-producing Esch erichia coli O157:H7. Proc. Natl. Acad. Sci. USA 2002, 99, 7669–7674.

43. Nishikawa, K.; Matsuoka, K.; Watanabe, M.; Igai, K.; Hino, K.; Hatano, K.; Yamada, A.; Abe, N.; Terunuma, D.; Kuzuhar a, H.; et al. Identification of the optimal structure required for a Shiga toxin neutralizer with oriented carbohydrates to fu nction in the circulation. J. Infect. Dis. 2005, 191, 2097–2105.

44. Watanabe, M.; Matsuoka, K.; Kita, E.; Igai, K.; Higashi, N.; Miyagawa, A.; Watanabe, T.; Yanoshita, R.; Samejima, Y.; T erunuma, D.; et al. Oral therapeutic agents with highly clustered globotriose for treatment of Shiga toxigenic Escherichi

a coli infections. J. Infect. Dis. 2004, 189, 360–368.

45. Kulkarni, A.A.; Weiss, A.A.; Iyer, S.S. Glycan-based high-affinity ligands for toxins and pathogen receptors. Med. Res. Rev. 2010, 30, 327–393.

46. Kulkarni, A.A.; Fuller, C.; Korman, H.; Weiss, A.A.; Iyer, S.S. Glycan encapsulated gold nanoparticles selectively inhibit Shiga toxins 1 and 2. Bioconjug. Chem. 2010, 21, 1486–1493.

47. Pohlentz, G.; Steil, D.; Rubin, D.; Mellmann, A.; Karch, H.; Müthing, J. Pectin-derived neoglycolipids: Tools for differenti ation of Shiga toxin-subtypes and inhibitors of Shiga toxin-mediated cellular injury. Carbohydr. Polym. 2019, 212, 323–3 33.

48. Detzner, J.; Gloerfeld, C.; Pohlentz, G.; Legros, N.; Humpf, H.U.; Mellmann, A.; Karch, H.; Müthing, J. Structural insight s into Escherichia coli Shiga toxin (Stx) glycosphingolipid receptors of porcine renal epithelial cells and inhibition of Stx-mediated cellular injury using neoglycolipid-spiked glycovesicles. Microorganisms 2019, 7, 582.

49. Hostetter, S.J.; Helgerson, A.F.; Paton, J.C.; Paton, A.W.; Cornick, N.A. Therapeutic use of a receptor mimic probiotic r educes intestinal Shiga toxin levels in a piglet model of hemolytic uremic syndrome. BMC Res. Notes 2014, 7, 331.

50. Lingwood, C. Verotoxin receptor-based pathology and therapies. Front. Cell. Infect. Microbiol. 2020, 10, 123.

51. Tzipori, S.; Sheoran, A.; Akiyoshi, D.; Donohue-Rolfe, A.; Trachtman, H. Antibody therapy in the management of Shiga t oxin-induced hemolytic uremic syndrome. Clin. Microbiol. Rev. 2004, 17, 926–941.

52. Arimitsu, H.; Sasaki, K.; Iba, Y.; Kurosawa, Y.; Shimizu, T.; Tsuji, T. Isolation of B subunit-specific monoclonal antibody c lones that strongly neutralize the toxicity of Shiga toxin 2. Microbiol. Immunol. 2015, 59, 71–81.

53. Moxley, R.A.; Francis, D.H.; Tamura, M.; Marx, D.B.; Santiago-Mateo, K.; Zhao, M. Efficacy of Urtoxazumab (TMA-15 h umanized monoclonal antibody specific for Shiga toxin 2) against post-diarrheal neurological sequelae caused by Esch erichia coli O157:H7 infection in the neonatal gnotobiotic piglet model. Toxins 2017, 9, 49.

54. De Macedo Henrique, I.; Sacerdoti, F.; Ferreira, R.L.; Henrique, C.; Amaral, M.M.; Piazza, R.M.F.; Luz, D. Therapeutic antibodies against Shiga toxins: Trends and perspectives. Front. Cell. Infect. Microbiol. 2022, 12, 825856.

55. Würzner, R.; Riedl, M.; Rosales, A.; Orth-Höller, D. Treatment of enterohemorrhagic Escherichia coli-induced hemolytic uremic syndrome (eHUS). Semin. Thromb. Hemost. 2014, 40, 508–516.

56. Salvadori, M.; Bertoni, E. Update on hemolytic uremic syndrome: Diagnostic and therapeutic recommendations. World J. Nephrol. 2013, 2, 56–76.

57. Pape, L.; Hartmann, H.; Bange, F.C.; Suerbaum, S.; Bueltmann, E.; Ahlenstiel-Grunow, T. Eculizumab in typical hemoly tic uremic syndrome (HUS) with neurological involvement. Medicine 2015, 94, e1000.

58. Walsh, P.R.; Johnson, S. Eculizumab in the treatment of Shiga toxin haemolytic uraemic syndrome. Pediatr. Nephrol. 2 019, 34, 1485–1492.

59. Mahat, U.; Matac, R.B.; Rotz, S.J. Use of complement monoclonal antibody eculizumab in Shiga toxin producing Esche richia coli associated hemolytic uremic syndrome: A review of current evidence. Pediatr. Blood Cancer 2019, 66, e2791 3.

60. Eaton, K.A.; Honkala, A.; Auchtung, T.A.; Britton, R.A. Probiotic Lactobacillus reuteri ameliorates disease due to entero hemorrhagic Escherichia coli in germfree mice. Infect. Immun. 2011, 79, 185–191.

61. Rund, S.A.; Rohde, H.; Sonnenborn, U.; Oelschlaeger, T.A. Antagonistic effects of probiotic Escherichia coli Nissle 191 7 on EHEC strains of serotype O104:H4 and O157:H7. Int. J. Med. Microbiol. 2013, 303, 1–8.

62. Cordonnier, C.; Thévenot, J.; Etienne-Mesmin, L.; Alric, M.; Livrelli, V.; Blanquet-Diot, S. Probiotic and enterohemorrhag ic Escherichia coli: An effective strategy against a deadly enemy? Crit. Rev. Microbiol. 2017, 43, 116–132.

63. Szu, S.C.; Ahmed, A. Clinical studies of Escherichia coli O157:H7 conjugate vaccines in adults and young children. Mic robiol. Spectr. 2014, 2, 2–6.

64. Rojas-Lopez, M.; Monterio, R.; Pizza, M.; Desvaux, M.; Rosini, R. Intestinal pathogenic Escherichia coli insights for vac cine development. Front. Microbiol. 2018, 9, 440.

65. Sabouri, S.; Sepehrizadeh, Z.; Amirpour-Rostami, S.; Skumik, M. A minireview on the in vitro and in vivo experiments w ith anti-Escherichia coli O157:H7 phages as potential biocontrol and phage therapy agents. Int. J. Food Microbiol. 201 7, 243, 52–57.

66. Lino, M.; Kus, J.V.; Tran, S.I.; Naqvi, Z.; Binnington, B.; Goodman, S.D.; Segall, A.M.; Foster, D.B. A novel antimicrobial peptide significantly enhances acid-induced killing of Shiga toxin-producing Escherichia coli O157 and non-O157 serot ypes. Microbiology 2011, 157, 1768–1775.

67. Puño-Sarmiento, J.; Anderson, E.M.; Park, A.J.; Khursigara, C.M.; Foster, D.E.B. Potentiation of antibiotics by a novel a ntimicrobial peptide against Shiga toxin producing E. coli O157:H7. Sci. Rep. 2020, 10, 10029.
68. Lingwood, C. Therapeutic uses of baterial subunit toxins. Toxins 2021, 13, 378.
69. Johannes, L.; Römer, W. Shiga toxins—From cell biology to biomedical applications. Nat. Rev. Microbiol. 2010, 8, 105–116.
70. Spooner, R.A.; Lord, J.M. How ricin and Shiga toxin reach the cytosol of target cells: Retranslocation from the endoplas mic reticulum. Curr. Top. Microbiol. Immunol. 2012, 357, 19–40.
71. Johannes, L. Shiga toxin—A model for glycolipid-dependent and lectin-driven endocytosis. Toxins 2017, 9, 340.
72. Sandvig, K.; Kavaliauskiene, S.; Skotland, T. The protein toxins ricin and Shiga toxin as tools to explore cellular mecha nisms of internalization and intracellular transport. Toxins 2021, 13, 377.
73. Li, D.; Selyunin, A.; Mukhopadhyay, S. Targeting the endosome-to-Golgi transport of Shiga toxins as a therapeutic strat egy. Toxins 2020, 12, 342.
74. Kouzel, I.U.; Kehl, A.; Berger, P.; Liashkovich, I.; Steil, D.; Makalowski, W.; Suzuki, Y.; Pohlentz, G.; Karch, H.; Mellman n, A.; et al. RAB5A and TRAPPC6B are novel targets for Shiga toxin 2a inactivation in kidney epithelial cells. Sci. Rep. 2020, 10, 4945.