Mechanisms of Signal Transduction in Haemolytic Uraemic Syndrome

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Introduction

Clinically, haemolytic uraemic syndrome (HUS) is marked by bloody diarrhoea, haemolysis, thrombocytopaenia, renal failure and occasional CNS lesions[1,2]. HUS is the leading cause of paediatric renal failure in the United States and is becoming increasingly common in Asia and South America[3]. This syndrome was first described in 1953, but little progress was made in characterizing its aetiology until a correlation between HUS and exposure to *E.coli* 0157:H7 was discovered in 1983[4]. Current opinion holds that HUS is mediated predominantly by a group of enterotoxins produced by the *E.coli* bacteria[5]. Enterotoxins that mediate HUS elevate intracellular concentrations of cAMP (cyclic adenosine triphosphate), cGMP (cyclic guanosine triphosphate), and other regulatory transduction messengers[6,7,8]. Studies indicate that the onset of HUS is induced by complex signal transduction pathways, which are initiated by toxin/plasma membrane interactions. Complexities associated with recognition of the specific pathogenic strain have hampered advances in clinical treatment of HUS. Therapies specifically targeting HUS do not exist; only supportive procedures including dialysis and blood transfusions are offered.

In this article I will discuss the mechanisms involved in diarrhoea induction. This is exceptionally important as it is generally thought that diarrhoea is the first clinical sign of HUS. Also, it is important to look at the induction of tissue damage and cell death since these complicate long term recovery of the patient. It is thought that targeting aspects of the signal transduction pathways in HUS may prevent the continuing destruction in the body.

Mechanisms of Diarrhoea Induction

As previously mentioned, *E.coli* infections have long been associated with HUS. *E.coli* 0157:H7 produces heat stable toxin (ST), heat labile toxin (LT), and verotoxins (VT)[7,9]. Gastrointestinal tissues are sensitive to all three toxins in spite of their chemical and physiologic differences. ST and LT cause secretory diarrhoea, while VT exposure results in renal failure, colonic ulceration, and endothelial cell death in humans[2,7,9].

ST is a peptide containing 18 to 19 amino acids and binds via a receptor/ligand mechanism[10]. The specific receptor associated with secretory diarrhoea is known as STaR (heat stable toxin receptor), and belongs to a family of membrane guanylate cyclases[11]. ST targets STaR localized on the apical membrane of intestinal cells[12]. Toxin/STaR coupling initiates a signal transduction cascade starting with the second messenger molecule cGMP and culminating in diarrhoea[10].

Early investigators theorized that activated cGMP stimulated cGMP-dependent protein kinases which in turn could phosphorylate and open cystic fibrosis transmembrane conductance regulator (CFTR) controlled chloride (Cl^-) channels[11]. These channels are located on the apical membrane of secretory epithelial cells. Activation of Cl^- channels, and the resulting chloride exodus, leads to an increase in salt and fluid secretion by the intestine, resulting in secretory diarrhoea. This simplistic pathway describing the onset of HUS is currently believed to be incomplete.

Forte[10] observed that murine (mouse) brush border cells treated with cGMP analogues are ineffective in stimulating Cl^- secretion in comparison with cAMP analogues. Since cGMP analogues are known to activate cGMP-dependent protein kinase, they proposed a mechanism for diarrhoeal induction that does not involve cGMP dependent kinase. Furthermore, he speculated that cGMP may cross-activate cAMP dependent protein kinase (PKA). An activated PKA pathway would lead to phosphorylation of CFTR Cl^- channels and thus induce diarrhoea. Additionally, Forte showed that PKA activity did indeed increase in response to ST, via intracellular cGMP.

This elegant mechanism simultaneously accounts for LT secretory diarrhoea. LT is known to activate intestinal adenylate cyclase, leading to increased intracellular cAMP[6,11]. PKA activity is upregulated in response to cAMP activation. Therefore, activation of cAMP-dependent protein kinase by both cGMP (ST induced) and cAMP (LT induced) would lead to essentially identical clinical symptoms. Since exposure to LT and ST result in secretory diarrhoea with similar electrolyte composition, it is postulated that these toxins act through a common pathway[6].

Forte's model was further strengthened when Hirayama[13] reported that the protein kinase inhibitors, isoquinolinesulfonamides, retard the usual fluid accumulation accompanying ST exposure. Hirayama also described an increase in phosphorylation of numerous proteins after cellular exposure to ST. This strongly supports Forte's claimed involvement of protein kinases in HUS.

The complexity of HUS makes it unlikely that only a single signal transduction pathway is involved. Gammell[6] proposed a supplemental mechanism in which ST triggers phospholipase A2 upon binding to glycolipid receptors. Phospholipase A2 mediates the release of arachidonic acid (AA) from membrane phopholipids. Arachidonic acid is metab-

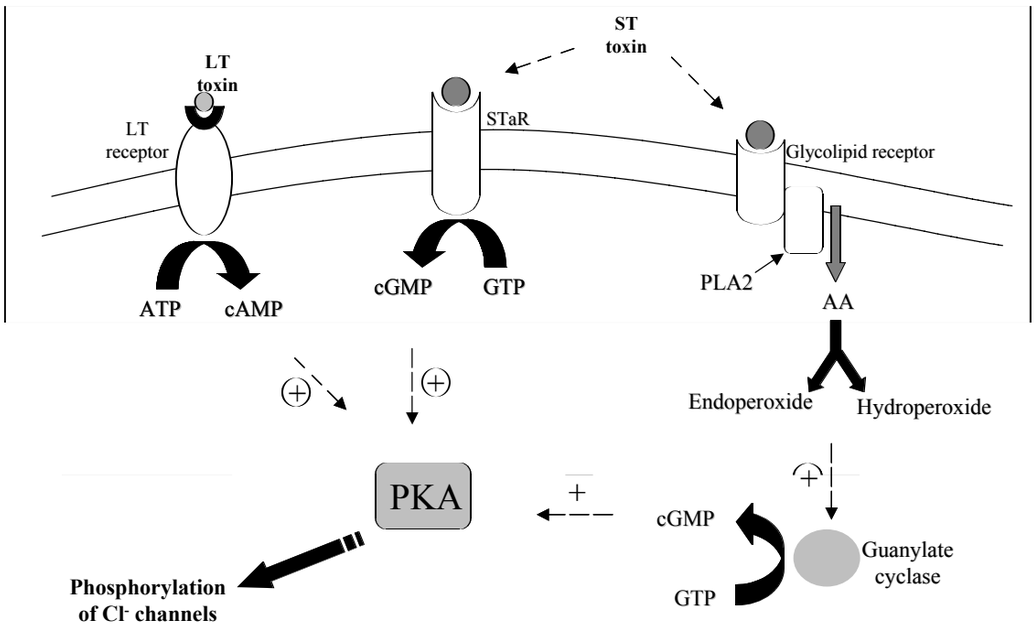

Figure 1: Diagram of the potential mechanisms of signal transduction involved in the action of LT and ST toxins.

Abbreviations: LT=heat labile toxin; ST=heat stable toxin; STaR=heat stable toxin receptor; PLA2=phospholipase A2; AA=arachidonic acid; PKA=protein kinase A; ATP=adenosine triphosphate; cAMP=cyclic adenosine monophosphate; GTP=guanosine triphosphate; cGMP=cyclic guanosine monophosphate

olized to endoperoxide or hydroperoxide, which are responsible for guanylate cyclase activation. The resulting increase in cytosolic cGMP could activate PKA via Forte's proposed pathway to initiate diarrhoea. Figure 1 illustrates the potential signal transduction pathways involved in the mechanism of action of ST and LT toxins.

The mechanism for HUS as proposed by Gemmell is by no means unrefuted. Dreyfus[14] found that control intestinal cells released AA at the same rate as ST treated cells. He reported no statistical differences between control and treated cells in free fatty acids, neutral lipids, or phospholipids. He also found no difference in the levels of the AA metabolites PGE2, PGF2-alpha, or thromboxane B2. Dreyfus's data seem to indicate that the arachidonic acid pathway may not be directly involved with the onset of HUS[14].

Induction of Tissue Damage and Cell Death

While ST and LT cause diarrhoea, verotoxin is an unusually potent exotoxin, which is responsible for the renal lesions, endothelial cell damage, CNS damage, and colonic ulceration associated with HUS[2]. VT is a heat labile toxin composed of two domains; the B-subunit binds to receptors at the membrane surface, and the A-subunit is responsible for the cytotoxic activity via inhibition of protein synthesis[15]. An N-glycosidase activity of the A-subunit, acting on the 28S rRNA of the 60S subunit, is responsible for this inhibition[16]. Prolonged protein inhibition results in cellular demise.

In addition to this well-defined mechanism of cell death, VT also induces, in cells expressing glycolipid globotriaosyl ceramide (Gb_3), morphological changes characteristic of apoptosis[16]. Only cells expressing Gb3 are sensitive to verotoxin[17]. Gb_3 is the main neutral glycolipid in human kidney and is particularly high in the renal cortex. Apoptosis is a normal physiologic form of cell death and is essential for the normal development of complex organisms. Cells undergoing apoptosis exhibit classic morphologic changes such as chromatin condensation and internucleosomal DNA cleavages[16]. Interestingly, tissues exposed to VT often exhibit these morphologic alterations indicative of apoptosis.

Several cell lines show toxin uptake and subsequent death in which protein synthesis is not inhibited[16]. Such cell lines show the two main characteristics of apoptosis: ultrastructural changes and cleavage of nuclear DNA. Gb_3 positive cells are therefore sensitive to verotoxin through two independent mechanisms; death results from protein synthesis inhibition and apoptosis.

Gb3 expression on the cell membrane is upregulated by exposure to inflammatory cytokines (tumor necrosis factor, interleukin-1 etc.) by 10-100 fold[18]. VT binding greatly increases in endothelial cells after cytokine exposure. Since cytokines are released in response to cellular injury, a positive feedback loop is initiated. VT binds to and kills tissue, upregulating cytokine release. The increased cytokine concentration enhances cellular sensitivity to the toxin.

Another family of VT receptors, P class antigens, are found on the surface of red blood cells and are highly homologous with Gb_3[2]. Taylor[15] noted that patients who are negative for, or only weakly express P1 antigens, experience severe HUS. Patients with high P1 expression have less severe symptoms. Based on these observations, Taylor

hypothesized that adsorption of free toxin onto erythrocytes may reduce the burden of VT to nucleated cells, which rely on RNA transcription. Since mature red blood cells have no nucleus and do not require protein synthesis, internalization of VT is not lethal.

Conclusion

HUS is a syndrome mediated by the enterotoxins produced by *E.coli* bacteria. Both heat labile and heat stable toxins are believed to cause secretory diarrhoea through cAMP-dependent protein kinase phosphorylation and activation of CFTR Cl^- channels. Verotoxin is especially cytotoxic and binds specifically to the Gb_3 receptor. VT mediates cell death through two mechanisms; apoptosis and inhibition of protein synthesis. Gb_3 is commonly expressed in endothelial tissue and the renal cortex; therefore endothelial and renal tissues are the most brutalised by VT. Future studies should concentrate on further elucidating the several signal transduction pathways involved in HUS, and focus on means of interfering with key aspects of its cytotoxic propagation. Development of specific antagonists against

References

1 Neumann M, Urizar R. Haemolytic uremic syndrome: current pathophysiology and management. *ANNA J* 1994;21:137-143.

2 Rose PE, Clark AJ. Haematology of the haemolytic uraemic syndrome. *Blood Rev* 1989;3:136-140.

3 Mizazaki S. The etiology and clinical features of haemolytic uremic syndrome. *Rinsho-Ketsueki* 1994;35:341-345.

4 Karmali MA, Steele BT, Petric M, Lim C. Sporadic cases of haemolytic uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *Lancet* 1983;1:619-620.

5 Karch H, Bockemuhl J. Infections by enterohemorrhagic *Escherichia coli*: a clinical and microbiologic problem and a challenge for the public health service. *Immuun Infekt* 1989;17:206-211.

6 Gemmell CG. Comparative study of the nature and biological activities of bacterial enterotoxins. *J Med Microbiol* 1984;17:217-235.

7 Hyun CS. Interaction of cholera toxin and *Escherichia coli* enterotoxin with isolated intestinal epithelial cells. *Am J Physiol* 1984;247:G623-631.

8 Guarino A, Cohen MB, Giannella RA. Small and large intestinal guanylate cyclase activity in children: effect of age and stimulation by *Escherichia coli* heat-stable enterotoxin. *Pediatr Res* 1987;21:551-555.

9 Guarino A, Guandalini S, Alessio M, Gentile G, Tarallo L, Capano G. Characteristics and mechanism of action of a heat-stable enterotoxin from infants with secretory diarrhoea. *Pediatr Res* 1989;25:514-518.

10 Forte LR, Thorne PK, Eber SL, Krause WJ, Freeman RH, Francis SH, Corbin JD. Stimulation of intestinal Cl^- transport by heat-stable enterotoxin: activation of cAMP-dependent protein kinase by cGMP. *Am J Physiol* 1992;C607-C615.

11 Kather H, Aktories K. The cAMP system and bacterial toxins. *Klin Wochenschr* 1983;61:1109-1114.

12 Carr S, Gazano H, Waldman SA. Regulation of particulate guanylate cyclase by *Escherichia coli* heat-stable enterotoxin: receptor binding and enzyme kinetics. *Int J Biochem* 1989;21:1211-1215.

13 Hirayama T, Masatochi N, Ito H, Takeda Y. Stimulation of phosphorylation of rat brush-border membrane proteins by *Escherichia coli* heat-stable enterotoxin, cholera enterotoxin and cyclic nucleatides, and its inhibition by protein kinase inhibitors, isophinolinesulfonamides. *Microbial Pathogenesis* 1990 ;8:421-431.

14 Dreyfus LA, Jaso L, Robertson DC. Characterization of the mechanism of action of *E. coli* heat-stable enterotoxin. *Infect Immun* 1984;44:493-501.

15 Taylor MC, Milford DV, Rose PE, Roy TC, Rowe B. The expression of blood group P1 in post-enteropathic haemolytic uraemic syndrome. *Pediatr Nephrol* 1990;4:59-61.

16 Mangeney M, Lingwood DA, Taga S, Caillou B, Tursz T, Wiels J. Apoptosis induced in Burkitt's lymphoma cells via Gb3/CD77, a glycolipid antigen. *Cancer Res* 1993;53:5314-5319.

17 Boyd B, Lingwood C. Verotoxin receptor glycolipid in human renal tissue. *Nephron* 1989;51:582.