Short Review on the Protection by Ouabain of E Coli Toxins

Amaral MM1, Girard MC1, Alvarez RS1, Paton AW2, Paton JC2, Repetto HA3, Sacerdoti F1 and Ibarra CA1

1Department of Physiology, Physiopathology Laboratory, Bernardo Houssay Institute of Physiology and Biophysics (IFIBIO Houssay-CONICET), Faculty of Medicine, University of Buenos Aires, Buenos Aires 1121, Argentina
2Department of Molecular and Cellular Biology, Research Centre for Infectious Diseases, University of Adelaide, Adelaide 5005, Australia
3Pediatrics Service, Alejandro Posadas Professor National Hospital, Buenos Aires 1684, Argentina

*Corresponding author: Horacio A Repetto, MD, Department of Pediatrics, Consultant Head Professor, Faculty of Medicine, University Buenos Aires, Alejandro Posadas Professor National Hospital, Argentina, Tel: 54 1147843237; Fax: 54 44690300; E-mail: harepetto@yahoo.com.ar

Received date: February 20, 2018; Accepted date: February 28, 2018; Published date: March 2, 2018

Copyright: ©2018 Amaral MM, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, reproduction, and re-distribution in any medium, provided the original author and source are credited.

Introduction

The most common form of hemolytic uremic syndrome (HUS) in children is associated with intestinal E Coli infection and the effect of its shiga toxin (Stx) on different types of cells. Diarrhea associated HUS is endemic in Argentina with the highest incidence rate in the world [1].

Once the systemic lesion is caused, management of the disease is symptomatic. So far, the only possibility to avoid the development of the lesions is by preventing either the infection or the deleterious action of the toxins.

Studies trying to obtain this goal have included the development of vaccines or antibodies against the bacteria or the toxins [2], and the inhibition of the generation of the toxin receptor (globotriosilceramide-GB3) [3,4].

Stx was the first toxin associated with HUS, described by Karmali et al. [5]. Paton and his group [6] described another toxin-subtilase AB in 2004. It has a different receptor (glycans) and induces proteolytic cleavage of an Endoplasmic Reticulum chaperone leading to cellular apoptosis like St does [7].

The authors of this paper have shown in studies in vitro that exposure of human glomerular endothelial cells [HGEC] [8] and proximal tubule epithelial cells (HK-2) [9] to both Stx2 and SubAB had decreased viability due to the induction of apoptosis. Stx2 has been shown to increase the expression of the pro-apoptotic factor Bax [10] and SubAB to induce a pathway depending of the pro-apoptotic proteins Bax/Bak [11].

Aperia and her group [12] in 2013 showed that the exposure of rat proximal tubular cells to Stx2 produced massive apoptosis with up regulation of the apoptotic factor Bax, increased cleaved caspase-3, and down regulation of the survival factor Bcl-xl. Co-incubation with ouabain (OUA) prevented all these effects. Moreover, they also showed that OUA reverse the imbalance between the two factors in mice treated with Stx2.

In this paper the authors performed studies in vitro with HGEC and HK-2 and with a mice model in vivo.

1. Firstly, they established non-cytotoxic concentrations of OUA in the cell cultures by developing cell viability assays. Concentrations above 30 nM were cytotoxic for both cell lines.

2. Then, they pre-incubated the cells with OUA for 24 h and then added either Stx2 or SubAB in the presence of OUA for another 48 h.

Inhibition of viability caused by both toxins was significantly lower in the cells treated with OUA. The maximum protective effect was obtained with OUA 20 nM.

3. They also studied morphologic alterations and cell detachment induced by both toxins. These induced edema, elongated shape, and detachment in the two cell lines and OUA prevented these morphological alterations. Changes can be observed in the paper by Amaral et al. [13].

4. In order to determine the possible mechanism of OUA protection, they determined apoptotic activity in both types of cells in the presence of both toxins. They found that the groups in which OUA was added had a significant decrease in the percentage of apoptotic and necrotic cells. Only the necrosis generated in HGEC by SubAB was not decreased.

5. Finally the authors also measured cell proliferation by cell count and IP-labeling and flow cytometry. Both toxins decreased cell proliferation and OUA prevented this effect.

6. Since OUA concentrations above the physiological range could affect the function of Na/K ATPase, the group evaluated this by measuring electrical current across HGEC and HK-2 monolayers. At the concentrations used in the experiments OUA did not modify the measurements, showing that the protective effect was not due to alterations on the function of the Na/K ATPase.

The results show that:

1. OUA at low concentration prevents the cytotoxic effect of Stx2 and SubAB on cultures of renal glomerular and tubular cells.

2. OUA also prevents morphologic alterations.

3. This protection is achieved by a decrease in the apoptotic and necrotic activity of both toxins.

4. The effect is not produced by interfering with the activity of the Na/K ATPase.

The laboratory is progressing with further experiments in animals, trying to replicate Aperia’s results.

We believe that these results and the actual evidences make it possible to proceed to the translation into human use.

The only drawback is that, in the experiments, OUA was added prior to the contact with the toxins.

In the clinic, the patients present diarrhea when first seen, and, supposedly, the toxin is already getting to its targets.
Conclusion

Nowadays, there is a PCR technique to rapidly determine the presence of Shiga toxin in the stools of a child with diarrhea. This may allow the rapid addition of OUA, hopefully before the microangiopathic lesion has been produced.

References

1. Rivas M, Chinen I, Miliwebsky E, Masana M (2014) Risk factors for shiga toxin-producing Escherichia coli-associated human diseases. Microbiol Spectr 2.
2. Bitzan M (2009) Treatment options for HUS secondary to Escherichia coli O157:H7. Kidney Int Suppl 112: S62-S66.
3. Silberstein C, Copeland DP, Chiang WL, Repetto HA, Ibarra C (2008) A glucosylceramide synthase inhibitor prevents the cytotoxic effects of Shiga toxin-2 on Human Renal Tubular Epithelial Cells. J Epithelial Biol Pharmacol 1: 71-75.
4. Silberstein C, Lucero M, Zotta E, Copeland DP, Lingyun L, et al. (2011) A glucosylceramide synthase inhibitor protects rats against the cytotoxic effects of Shiga toxin 2. Pediatr Res 69: 390-394.
5. Karmali MA, Petric M, Lim C, Fleming PC, Arbus GS, et al. (1985) The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing Escherichia coli. J Infect Dis 151: 775-782.
6. Paton AW, Srimanote P, Talbot UM, Wang H, Paton JC (2004) A new family of potent AB(5) cytotoxins produced by shiga toxigenic Escherichia coli. J Exp Med 200: 35-46.
7. Paton AW, Beddoe T, Thorpe CM, Whisstock JC, Wilce MC, et al. (2006) AB5 subtilase cytotoxin inactivates the endoplasmic reticulum chaperone BiP. Nature 443: 548-552.
8. Amaral MM, Sacerdoti F, Jancic C, Repetto HA, Paton AW, et al. (2013) Action of shiga toxin type-2 and subtilase cytotoxin on human microvascular endothelial cells. PLoS ONE 8: e70431.
9. Marquez LB, Velazquez N, Repetto HA, Paton AW, Paton JC, et al. (2014) Effects of Escherichia coli subtilase cytotoxin and shiga toxin 2 on primary cultures of human renal tubular epithelial cells. PLoS ONE 9: e87022.
10. Jones NL, Islur A, Haq R, Mascarenhas M, Karmali MA, et al. (2000) Escherichia coli shiga toxins induce apoptosis in epithelial cells that is regulated by the bcl-2 family. Am J Physiol Gastrointest Liver Physiol 278: G811-G819.
11. May KL, Paton JC, Paton AW (2010) Escherichia coli subtilase cytotoxin induces apoptosis regulated by host bcl-2 family proteins bax/bak. Infect Immun 78: 4691-4696.
12. Burlaka I, Liu XL, Rebetz J, Arvidsson I, Yang L, et al. (2013) Ouabain protects against shiga toxin-triggered apoptosis by reversing the imbalance between Bax and Bcl-xL. J Am Soc Nephrol 24: 1413-1423.
13. Maria MA, Magali CG, Romina SÁ, Adrienne WP, James CP, et al. (2017) Ouabain Protects Human Renal Cells against the Cytotoxic Effects of Shiga Toxin Type 2 and Subtilase Cytotoxin. Toxins (Basel) 9: 226.