Neutralizing activity of Gb3 analog-conjugated copolymers on cytotoxicity of Shiga toxins

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Shiga toxins (Stxs) produced by *Escherichia coli* (STEC) cause systemic vascular damage, manifested as hemolytic uremic syndrome in humans, via the specific recognition of galactobiosyl α (1-4) linkage in globosyl Gb_2- and Gb_3- ceramides (Cer) on target endothelial cells. For the prevention of Stxs-Gb_3Cer binding, various approches have been reported. One of these is to generate analogs of the natural receptor which are able to compete with Gb_3Cer on endothelial cell surface to prevent systemic Stxs recognition. In these attempts, water soluble glycoconjugate polymers carrying carbohydrate segments related to Gb_3 or Gb_2, were synthesized and subjected to biological assay. Stx1 and Stx2 neutralization assay using HeLa cells was performed for the acrilamide copolymers: (1) Gb_3-1-*O*-aryl, (2) Gb_2-1-*O*-aryl, (3) galacto-trehalose type Gb_2-6-*O*-alkyl, (4) lactose-1-*O*-aryl, (5) α-galactose-1-*O*-aryl, (6) galacto-trehalose-4-*O*-alkyl, (7) trehalose-6-*O*-alkyl copolymers. Copolymer solutions (50μM) were mixed with either crude Stx1 or Stx2 (0.05-0.2 units) and incubated at 37 °C for 1h. Then the copolymer-Stxs mixtures (20μl) were added to HeLa cells and incubated in 5% CO_2 at 37°C for further 48 h. The number of living cells was determined by using AlamarBlue. After 2-h incubation, fluorescence was determined at 530 nm excitation and 590 nm emission. Cells not exposed to toxin and cells exposed to either Stx1 or Stx2 were prepared on each plate as respective controls. The assay has revealed that galacto-trehalose type Gb_2-6-*O*-alkyl copolymer (3) possess blocking activity against Stx1 slight lower than that of the natural types Gb_3-1-*O*-aryl (1) and Gb_2-1-*O*-aryl (2) copolymers, but higher than that of theαgalactose-1-*O*-aryl copolymer (5) and lactose-1-*O*-aryl copolymer (4). Galacto-trehalose type Gb_2-6-*O*-alkyl copolymer (3) was found to have weak or no activity against Stx2. Galacto-trehalose-4-*O*-alkyl copolymer (6) showed no activity against Stx1 nor against Stx2. Moreover trehalose-6-*O*-alkyl copolymer (7) showed weak activity against Stx1 but not Stx2. In conclusion, galacto-trehalose type Gb_2-6-*O*-alkyl copolymer (3) showed a potential utility for the development of sugar-based therapeutic agents against Stxs, although its activity is weaker than those of the natural type compounds (1) and (2).