Low-Level Release of Shiga-Like Toxin (Verocytotoxin) and Endotoxin from Enterohemorrhagic Escherichia coli Treated with Imipenem

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Shiga-like toxin (SLT) and endotoxin may participate in the pathogenesis of enterohemorrhagic Escherichia coli (EHEC) infection. Levels of release of SLT and endotoxin from EHEC treated in vitro with antibiotics were estimated. There were differential levels of release of SLT and endotoxin from EHEC treated with different antibiotics. Treatment of EHEC strains, namely, E. coli O157, O111, and O26, with imipenem induced much lower levels of release of SLT and endotoxin than treatment with ceftazidime.

Enterohemorrhagic Escherichia coli (EHEC) produces Shiga-like toxin (SLT), and EHEC infection causes hemolytic-uremic syndrome (HUS), which is the leading cause of acute renal failure in young children (3, 7, 8). The low-level release of SLT in EHEC infection may be important in prevention of the disease. Endotoxin liberated from EHEC may also participate in the development of EHEC-associated diseases, such as HUS. Recently it was reported that the release of endotoxin is dependent on the kind of antibiotics used (1, 2, 4, 9). β-Lactam antibiotics are considered the antibiotics most responsible for the liberation of excessive amounts of endotoxin. It has been reported that differences in the propensities of β-lactam antibiotics to release lipopolysaccharide exist among and within the subclasses. Of the many β-lactams studied, imipenem (IPM), a member of the carbapenem antibiotic subclass, has been shown to cause the release of lower amounts of endotoxin during bacterial exposure (2, 5, 6, 11). However, there are few reports on the release of SLT from EHEC by antibiotic treatment. In this study, we compared the levels of release of SLT and endotoxin from EHEC treated in vitro with IPM and ceftazidime (CAZ). Here, we describe the low-level release of SLT and endotoxin from EHEC by IPM.

CAZ and IPM were obtained from Tanabe Pharmaceutical Co. and Banyu Pharmaceutical Co., respectively. Their stock solutions were prepared by the methods recommended by the manufacturers. Clinical isolates of E. coli O157:H7, O111, and O26 were used. The MICs of CAZ and IPM for those E. coli strains were 0.63 and 1.25 μg/ml, respectively. Briefly, a single colony was selected, suspended in L broth (5 ml), and placed in a test tube with shaking at 37°C overnight. The bacterial suspension (approximately 10 μl) was diluted in Mueller-Hinton broth (1 ml) and cultured for 2 h with shaking. Log-phase bacteria (approximately 10⁵ CFU/100 μl) were added to each 1 ml of decomplemented fetal calf serum containing various MICs of IPM or CAZ. Endotoxin-free fetal calf serum (Bio Whittaker, Walkersville, Mass.) was decomplemented by heating for 30 min at 56°C and used for the experiment. These mixtures were incubated with shaking for 8 h at 37°C. After incubation, the bacterial suspensions were passed through a 0.22-µm-pore-size filter and the filtrates were collected. Levels of release of SLT-I and -II were determined with an SLT-I and -II detection kit by latex bead agglutination (Denka Seiken, Tokyo, Japan), which can detect SLT at 1 to 2 ng/ml. Briefly, the sample solution was subjected to serial twofold dilution, treated with latex beads coated with anti-SLT-I or -II antibody in microplates, and left overnight at room temperature. The levels of SLT-I and -II in the samples were expressed as the maximal dilutions showing positive agglutination. The levels of release of SLT-I and -II from EHEC treated with IPM and CAZ were compared (Table 1). Treatment of E. coli O157 with IPM at concentrations two and four times its MIC for 8 h caused much lower levels of release of SLT-I than treatment with CAZ at similar multiples of its MIC. Treatment with IPM also showed lower levels of release of SLT-II than those with CAZ. However, there was no significant difference in levels of SLT-I and -II release from E. coli O157 treated with IPM and CAZ at one-half their MICs. Low-level release of SLT with IPM was also seen in SLT-I- and SLT-II-producing E. coli O111 and SLT-I-producing E. coli O26. Further, the time course of the release of SLT-I from E. coli O157 by treatment with IPM and CAZ at two times their MICs was monitored (Fig. 1). The release of SLT-I by IPM reached its maximal value 2 h after the treatment and did not increase thereafter. On the other hand, the treatment with CAZ increased its release gradually up to 6 h.

Next, levels of endotoxin release from E. coli O157, O111, and O26 treated with IPM and CAZ were compared (Table 2). Endotoxin levels were determined with Endospecy ES-6 and the Toxicolor system DIA-MP (Seikagaku Corp., Tokyo, Japan) as instructed by the manufacturer. The means of results from three dishes with standard deviations were determined. Treatment with IPM at one-half, two, and four times the MIC resulted in 5- to 60-fold-lower levels of release of endotoxin into the culture fluid than those with CAZ. As shown in Fig. 1, the release of endotoxin increased markedly after the treatment with CAZ and reached a peak level 4 h after the treatment with CAZ. On the other hand, IPM increased it only slightly. Morphological changes of E. coli O157 bacteria treated with IPM and CAZ were studied. There were marked differences in the resulting bacterial morphologies. IPM treatment induced rod-shaped bacteria to become rounded, whereas exposure to CAZ induced the formation of long filaments (data not shown). In addition, treatment with levofloxa-
cin, a new quinolone, resulted in high levels of release of SLT and endotoxin and filament formation similar to that seen with CAZ (data not shown).

This study shows that IPM and CAZ differentially induce SLT and endotoxin release from EHEC and that IPM liberates lower amounts of SLT and endotoxin than CAZ. While endotoxin is lipopolysaccharide present on the outer membranes of gram-negative bacteria, SLT-I and -II are heterodimeric protein toxins that may be localized or secreted differentially. This is the first report that IPM may result in low-level release of SLT-I and -II from EHEC. This finding is of interest, because SLT-I is the predominant toxin present in cell lysates whereas SLT-II may be present in a much higher titer than SLT-I in the culture supernatant (10). IPM might not inhibit the release of SLT-II from bacteria, because it is secreted into the culture supernatant. Therefore, the inhibition of the release of SLT-I and -II by IPM might be due to different mechanisms. The low-level release of SLT-I from IPM-treated EHEC was consistent with the low-level release of free endotoxin by IPM. Both SLT-I and endotoxin are cell-associated toxins, and there is little spontaneous release of either from bacteria. Low-level release of endotoxin by IPM has already been reported by several investigators (2, 5, 6, 11).

It is likely that low-level release of SLT-I may be related to antibiotic-induced morphological changes. EHEC organisms treated with IPM became round cells (spheroplasts), whereas those treated with CAZ formed long filaments. It has been reported that the release of endotoxin is closely related to morphological changes observed during antibiotic treatment (i.e., spheroplast and filament formation) (5, 6, 9, 11). Based on the fact that SLT-I is predominantly the cell-associated toxin, low-level release of SLT by IPM may be associated with the formation of round cells.

Differential levels of release of endotoxin and SLT between bacteria treated with IMP and those treated with CAZ suggested that antibiotic treatment of EHEC infection might influence prognosis. The excessive release of SLT and endotoxin by treatment with antibiotics might be one of contributing factors to mortality and morbidity in EHEC infection. The inhibition of dual release of SLT and endotoxin by IPM treatment might reduce their participation in the development of EHEC-associated diseases, such as HUS. It remains to be further clarified whether our findings can be applied to antibiotic selection for patients with EHEC infection.

REFERENCES