HEMOLYSIN FROM *escherichia coli* INDUCES OXIDATIVE STRESS IN BLOOD

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INTRODUCTION

Oxidative stress is caused by an imbalance between the production of oxidants and the levels of antioxidants present in the biological system. In this situation, the overproduction of reactive oxygen species (ROS) can lead to the damage of cellular components including lipids, protein, and DNA. If this damage is not repaired, mutagenesis and cellular death can occur, and participate in the pathogenesis of different diseases such as Chagas, meningitis and illnesses associated with *Escherichia coli* infections such as hemolytic uremic syndrome and pyelonephritis (1). *E coli* is an important food-borne pathogen in Argentina and other parts of the world. During the infection, this bacterium secretes different products, including lipopolysacharide, Shiga toxin and Hemolysin (HlyA). The latter is a pore-forming toxin and numerous effects on different cellular populations have been attributed to sublytic concentrations of this toxin, including secretion of ROS and nitric oxide (2,3).

Therefore, considering that oxidative stress has been linked to *E. coli* infections and that HlyA is a very important virulence factor of this bacterium, the objective of this study was to analyze the capacity of this toxin to induce oxidative stress in whole blood cultures (WBCs), which could contribute to the pathogenesis of the infection by this pathogen.

MATERIALS AND METHODS

The capability of HlyA, purified from a clinical isolate of *E. coli* by gel chromatography, to generate ROS was examined in WBCs using luminol sensitized chemiluminescence. In addition, to determine whether ROS production by HlyA resulted in oxidative damage of plasma proteins and lipids, whole blood were incubated with an equal volume of HlyA (0.4 or 0.2 hemolytic activity (HU)/ml) or PBS like negative control for 4 h at 37°C. After incubation, the blood samples were centrifuged and the plasma obtained was assayed for different oxidative stress biomarkers, such as malonyldialdehyde (MDA), carbonyl residues and advanced oxidation protein products (AOPP). The antioxidant system also was evaluated through the determination of total antioxidant capacity of plasma by the Ferric Reducing Antioxidant Power (FRAP) assay and the activity of superoxide dismutase (SOD) assayed photochemically based on the inhibition of nitroblue tetrazolium reduction.

RESULTS

We found that HlyA increased the level of free radicals detected by chemiluminescence assay. Moreover, plasma MDA levels, as an index of lipid peroxidation, were significantly increased in cultures treated with HlyA in comparison with those found in control cultures. In addition, biomarkers of protein damage such as carbonyl residues and AOPP also were elevated after treatment of blood with HlyA. On the other hand, a decrease in total antioxidant capacity of plasma and in the activity SOD was observed in plasma from blood treated with HlyA.

CONCLUSIONS

Collectively, our data demonstrate that low concentrations of *E. coli* hemolysin induce oxidative stress in WBCs. This oxidative imbalance produced by HlyA may have an important role in the pathogenesis of infections caused by *E. coli* strains that produce this toxin.

REFERENCES


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