STRUCTURE OF ENDOTHELIAL CELLS IN HEALTH AND DISEASE:

Intestinal microvascular response to diarrhoeal disease

Abstract

The intestinal microvasculature plays a central role in the absorptive and secretory functions of the small and large intestine and changes in the microcirculation contribute to the pathophysiology of diarrhoeal diseases. Shiga toxin producing *Escherichia coli* (STEC) has been recognized as a major cause of large scale epidemics and produces severe bloody diarrhea. Rotavirus is an important cause of severe gastroenteritis and diarrhoea in infants and young children worldwide and is associated with mortality in developing countries. This work has been undertaken because ultrastructural changes produced in intestinal microvascular endothelial cells in Shiga toxin *E.coli* infection, rotavirus infection and rotavirus toxin NSP4 infection are not known.

In this study, 22 adult monkeys for STEC infection, 18 five- day old infant rats for rotavirus infection and 21 five–day old infant rats for rotavirus toxin NSP4 infection have been used. Four control monkeys were injected with nonpathogenic E.coli and 23 control rats were injected with sterile saline. Animals were sacrificed at prescribed time points, tissue samples were taken from the ascending colon, mid colon and descending colon of monkeys and proximal and distal small intestine, caecum and colon of infant rats. Tissues were fixed in 3% glutaraldehyde, post fixed in 1% osmium tetraoxide, dehydrated in ascending grades of alcohol and embedded in epoxy resin. Semithin sections were taken, stained with toludine blue and observed under light microscope. Then ultra thin sections were taken and stained with uranyl acetate and lead citrate and observed under electron microscope. In each section, arterioles, capillaries and venules in the mucosa and sub-mucosa were photographed. The endothelial changes quantified were ruffling of the luminal membrane, vacuolation of cytoplasm, swelling of endothelial cell, swelling of cell organelles, rarefaction of cytoplasm and disruption of cytoplasm. To estimate the frequency at which these changes occur, the number of vessels showing these changes were counted and expressed as a percentage of the total number of vessels in that segment at that time point. A new program written in JAVA was used to prove

the endothelial swelling and ruffling of the luminal surface of the capillaries of STEC treated and control monkeys.

The microvasculature of the intestines in control animals was mostly normal. But few vessels showed endothelial changes and no cytoplasmic rarefaction and distruption were seen. The intercellular junctions in all blood vessels were tight and the lumen was free of stagnant blood cells and cell fragments.

In STEC treated monkeys, endothelial changes were most marked in the capillaries, but also in the venules and less obvious in the arterioles. Damage was most pronounced at 24 hours, although occurred at 6 hours and persisted upto 12 days post infection. Our findings also indicate that the microvasculature of the ascending and mid colon was affected more than the descending colon.

Size of the capillary lumen in infected monkeys was less than the controls and this difference was statistically significant. Lumen of control monkeys was more smooth than that of infected monkeys and this difference was statistically significant. This new way of quantification confirms that endothelial cell swelling and ruffling of the luminal membrane occur in colonic microvasculature of monkeys infected with STEC at 6 and 24 hours post infection.

In Rota virus infection, the significant damage was seen in arterioles of distal small intestines. Capillaries were severely damaged. The changes were more prominent in the colon. Damage to venules was more in the caecum and colon. These changes were marked by 12 hours post infection in all type of vessels and extended upto 144 hours.

In Rota virus toxin NSP4 infection, the changes were more significant in arterioles of colon. Evidence of endothelial damage was more in capillaries. Over all, the changes in caecum were most prominent. In venules, endothelial changes were more prominent in proximal small intestine. These changes began at 4 hours in all type of vessels and persisting till 48 hours post infection.

The microvascular changes in our study would be useful in the further study of pathogenic mechanisms and prevention methods in STEC infection, Rotavirus infection and Rotavirus toxin NSP4 infection.