

3071 Paracellular Ion Selectivity Parallels Loss of Mucosal Barrier Following Irradiation

P. Vijaygopal, L. Yin, S. Mohankumar, M. Zhang, W. O'Dell, L. Zhang, P. Okunieff, S. Vidyasagar, *UF Shands Cancer Center, Gainesville, FL*

Purpose/Objective(s): Tight junctions formed by the mature enterocyte are an efficient barrier with the necessary machinery for electrolyte and nutrient absorption. Radiotherapy targets rapidly dividing cells of malignant tumors and affects the epithelial cells of the gastrointestinal (GI) tract causing epithelial barrier dysfunction. We have shown that irradiation (IR) results in anion secretion and defective Na-coupled glucose transport (SGLT1) leading to diarrhea, which often resolves in 10-14 days. However, little is known about IR-induced alterations in paracellular permeability and ion selectivity and the time required for its restoration.

Materials/Methods: Swiss mice were exposed to IR. Time dependent changes in permeability were determined on days 3 or 14. Relative permeability of Cl⁻ and Na⁺ (P_{Cl}/P_{Na}) was determined using the modified Goldman-Hodgkin-Katz equation and compared to the plasma endotoxin level, conductance measured using an Ussing chamber, and changes in tight-junction protein levels determined in Western blots.

Results: The membrane selectivity ratio measured on day 3 varied from 0.52 ± 0.1 with Na⁺ ions more permeable than Cl⁻ in non-IR mice; 0.71 ± 0.2 in 5-Gy mice; and 0.78 ± 0.2 in 7-Gy mice, which showed decreased selectivity. The selectivity ratio (P_{Cl}/P_{Na}) measured on day 14 after IR showed 0.49 ± 0.1 for non-IR mice; 0.59 ± 0.1 for 5-Gy mice; and 0.67 ± 0.2 for 7-Gy mice. This result suggests restoration of ion selectivity at 5 Gy and not at 7 Gy. Similarly, conductance decreased significantly with time in 5-Gy mice (42 ± 4.6 vs 29.3 ± 3.1) but not in 7-Gy mice (52.7 ± 0.4 vs 49 ± 0.43). Plasma endotoxin levels corresponded to changes in paracellular permeability and conductance changes and IR dose. Levels were 0.614 ± 0.2 at 0 Gy; 41.186 ± 0.6 at 5 Gy; and 63 ± 0.7 EU/ml at 7 Gy. With time, endotoxin levels showed a significant decrease in 5-Gy mice but not in 7-Gy mice (16.3 ± 2.1 vs 51 ± 6.7). The Western blot for tight-junction proteins showed that claudin-1 and JAM-A were increased in 3-Gy or 5-Gy mice, while protein levels decreased in 7-Gy mice.

Conclusions: IR-induced changes in the paracellular selectivity of ions, through alterations in the tight-junction framework, correlate with changes in conductance and plasma endotoxin levels in both a dose and time dependent manner. Our results show that IR-induced alterations in paracellular permeability and barrier function occur with increasing IR dose. The lower dose (5 Gy) showed restoration of the barrier function with time. Similar restoration was not seen at a higher IR dose (7 Gy), suggesting irreversible structural changes in the tight-junction framework with continued compromise in barrier function. Therefore, it is essential to give supportive therapy to patients receiving high-dose IR to prevent long-term complications.

Author Disclosure: P. Vijaygopal: None. L. Yin: None. S. Mohankumar: None. M. Zhang: None. W. O'Dell: None. L. Zhang: None. P. Okunieff: None. S. Vidyasagar: None.