Effects of radiation on human brain endothelial barrier function
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The human brain vasculature is crucial to healthy functioning of the brain and its dysfunction is not only a primary event in a range of neurodegenerative diseases but also an important influencing factor in many others. Furthermore, vessel pathologies involve common susceptibility factors such as oxidative stress and inflammation so any radiation effects on capillaries can be influenced by other preexisting conditions.

Here we have examined the effects of radiation on BBB models using a variety of endpoints to assess barrier function. These include transendothelial electrical resistance (TEER), morphological effects, localization of adhesion and cell junction proteins, and permeability of molecules through the endothelial barrier.

Development of BBB models.
TEER measurements can be carried in real time and can be monitored continuously. We therefore used TEER measurements to optimize the culture conditions for highest resistance. Human Brain Microcapillary endothelial cells (HBMEC) were used to represent human brain vasculature and pooled Human Umbilical Vein Endothelial cells (HUVEC) were used for a comparison and to represent human endothelial barriers in general. Conditions were established for a highly impermeable barrier. Resistance for HBMEC reached ~ 1900 Ω and for HUVECs a lower resistance of ~ 1600 Ω. Other endpoints support an impermeable barrier. Cells are tightly held together by Cell Adhesion Molecules, Adherens junctions, and Tight junctions in 2-D and in 3-D capillary models. Permeability of 70kDa and 10kDa molecules was low enough to measure rates over a period of 24 hours.

Effects of Gamma irradiation.
To develop and optimize the assays for efficiency at NSRL we carried out experiments using gamma rays. These studies also have relevance to radiotherapy treatments since effects were seen at the fractionated radiotherapy dose of 2Gy. TEER measurements showed a clear and reproducible response. 2-4 hours after irradiation there was a transient drop in resistance in both cell types. HUVEC and HBMEC respond with similar kinetics and magnitude although brain cells remain consistently higher than HUVEC. After 6 hours, resistance returned to normal. The effect was dose dependent, a higher dose gave a greater drop in resistance.

Fixation and visualization of parallel barrier cultures showed that at 3 hours (lowest point for resistance) a proportion of cells (> 5%) had detached leaving gaps in the monolayer. At 6 hours the remaining cells had migrated and spread to reform a continuous barrier.

Visualization of various junction and adhesion proteins showed no great change in the actin cytoskeleton, adherens junctions, or in tight junction protein localization. The cell adhesion molecule - Platelet endothelial cell adhesion molecule (PECAM) however, became absent between cells at the time of low resistance. Experiments utilizing the fact that homophilic binding of PECAM is reduced at low temperatures, were carried out. Gamma radiation caused further uncoupling of PECAM suggesting that heterophilic binding is affected by Gamma radiation. This transient opening of the endothelial barrier was not detected using transwell inserts and the passage of labeled molecules of sizes 10kDa and 70kDa through the barrier. However, measurements over 24 hours show a longer-term increase in permeability after exposure to gamma radiation.

In conclusion, gamma photons cause a transient permeability of the endothelial barrier characterized by a loss of PECAM but not junction proteins. Although the damage is repaired there is a weakening of the barrier, which shows an increase in permeability over 24 hours.

Effects of ion particles.
Experiments with ion particles are ongoing although preliminary results show that high and low LET particles affect endothelial barriers in a different way to photon radiation. Transwell experiments show a greater increase in permeability after exposure to 2 and 1Gy of high-energy protons and Fe ions respectively. In addition, both ion particles cause a disruption of the tight junction protein ZO-1. These preliminary results will be confirmed and expanded during the summer run.

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