Effects of ionizing radiation on the neurovascular unit: an *in vitro* study

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Radiation therapy is commonly used for the treatment of both primary and metastatic tumors of the CNS but it is well established that it also induces dramatic side-effects due to radiation damages to normal brain tissue. Acute, early delayed and late delayed reactions have been described and endothelial, glial, neuronal cell loss as well as microenvironmental alterations (neuro-inflammation, hypoxia…) are associated to the emergence of brain radiation injuries.

In the present study, we have focused our attention on the effects of ionizing radiation on the brain microvasculature by the use of an *in vitro* reconstituted blood-brain barrier (BBB) developed from cocultures of rat primary glial cells and bovine brain capillary endothelial (BBCE) cells (Dehouck MP etal., 1990, 1992). On this model, the effects of gamma radiation were observed on the cellular density of endothelial and glial cells by nuclear staining and counting. Tight junction (TJ) proteins (ZO-1, ZO-2, occludin, claudine 5) and F-actin were detected by immuno-cytochemistry and the permeability of the endothelial monolayer to [^{14}C]-sucrose, fluorescein and various dextrans was determined until 8 days after radiation exposure. DNA double-strand breaks were also estimated by phospho-H2A.X immunostaining. Using real-time quantitative PCR, mRNA expression was determined for ZO-1, ZO-2, occludin, caveolin-1, clathrin B and caspase 3 isolated from BBCE cells and for ICAM-1 and IL1-β isolated from glial cells. At least, the protective action of NAC, SOD, nifedipine, EPO and dexamethasone on the permeability of irradiated BBCE cells was assessed.

Our results show that gamma irradiation can induce an increase in paracellular permeability of [^{14}C]-sucrose, fluorescein and dextrans but without major alteration of TJ-protein labelling. A progressive decrease of the number of BBCE and glial cells as well as long-lasting DNA damages are observed after gamma irradiation. mRNA analysis revealed overexpression of caspase-3, ICAM-1, IL1- β and a reduction of ZO-1 and occludin expression compared to unirradiated cells. A protective action of dexamethasone on the paracellular permeability was observed in the first days after exposure.

These results confirmed *in vivo* experiments that showed alterations of the BBB after radiation exposure and validate this *in vitro* model of BBB as a useful tool to elucidate the effects of ionizing radiation on the different components of the neurovascular unit. A better understanding and awareness of this phenomenon are essential for designing appropriate treatment modality in brain radiotherapy or accidental overexposure.

References

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