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DISSERTATION SUMMARIES

Evaluation of the effect of unsaturated fatty acids and irradiation on U87 glioma cell line

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Glioma is an invasive, aggressive form of brain tumors, with high rate of recurrence and resistance to radio and chemotherapy. Patients usually survive less than two years after diagnosis. The common treatment consists of surgical resection, followed with radiotherapy and/or chemotherapy. There is a great necessity for development of new therapeutic methods. Strategies are under development or clinical phase.

UFAs (unsaturated fatty acids) are one of the adjuvants that are applied as therapeutic agents for the treatment or alleviation of the symptoms of several diseases, like diabetic retinopathy, insulin resistance, inflammatory Bowel disease, cardiovascular diseases and several types of cancer. Numerous *in vitro* and *in vivo* studies prove the benefic effect of application of PUFAs (polyunsaturated fatty acids) as agents (solely or combined with chemo- or radiotherapy) in glioma therapy. Application of γ-linolenic acid on human glioma in clinical phase inhibited recurrence.

In order to determine the ideal type and concentration of UFAs as adjuvants in radiotherapy, we performed *in vitro* evaluation on U87 MG glioma cell line. We evaluated the effect of the following UFAs: arachidonic acid (AA, 20:4n-6), docosahexaenoic acid (DHA, 22:6n-3), gamma-linolenic acid (GLA, 18:3n-6), eicosapentanoic acid (EPA, 20:5n-3) and oleic acid (OA, 18:1n-9). Cells were treated with each fatty acid solely and in combination with 5 or 10 Gy.

We performed biochemical (LDH and MTS) and biophysical (RT-CES) assay to evaluate the effect of these fatty acids. We found that AA, DHA and GLA was more effective in sensitizing U87 cells to radiotherapy, so further experiments were performed with these three compounds, namely morphological, gene and miRNA expression analysis.

Statistical analysis of more than forty parameters based on holographic images was performed. Cell number and confluence was significantly diminished when they were treated with AA or when they were exposed to 10 Gy combined with AA, DHA or GLA. Application of PUFAs as adjuvants to 10 Gy caused significant alteration in cell thickness and irregularity, which indicate that cells have rounded and detached from the surface.

The molecular pathways that influence and determine the course of glioma when it is treated with UFAs (solely or in combination with radiotherapy) are not entirely deciphered yet. Thus, we decided to investigate the effect of AA, DHA and GLA at gene and miRNA expression level. Based on the scientific literature we have chosen to investigate gene expression on U87 cells that were solely PUFA treated or irradiated or co-exposed to PUFA and 10 Gy. We noticed significant alteration for at least one of these parameters in expression of endoplasmatic reticulum stress related genes (Grp78, DDIT3); genes which respond to oxidative stress (HMOX1, AKR1C1, NQO1), oncogenes (p53, c-Myc); early response genes (Egr1, TNF- , FOSL1, c-Fos); Gadd45a - a validated target in cancer treatment - and Notch1, a potential therapeutic target in glioblastoma. Out of the oxidative stress responsive genes that responded significantly to co-exposure to PUFA and 10 Gy HMOX1 is a potential target in glioma treatment, and NQO1 is a priority one.

Due to their small size and stability the study of the effect of miRNA on glioma therapy is an intensively investigated field (Low et al., 2014). We investigated the effect of AA, DHA, GLA and/or 10 Gy on U87 cells for the following miRNAs: miR34a, miR96, miR146, miR181a, miR148a, miR148b and miR152. Significant effect was noticed in case of miR146 and miR181a.

Our gene expression studies indicate that GLA and irradiation alter the expression of the therapeutic target Notch1 significantly. When 10 Gy is combined with AA, but not with DHA or GLA, changed the expression of several genes in a significant manner (p53, c-Myc, TNF-α and c-Fos). Our results confirm that UFAs are potent agents which enhance the effectiveness of radiotherapy.

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Synaptic changes in depression disorders

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Major depressive disorder (MDD) is predicted to become the leading cause of disability worldwide by the year 2030, representing an enormous financial and social burden. Clinical management of MDD is quite limited due mostly to the fact that the neurobiology of depression and the mechanisms of antidepressant therapy are still largely unknown.

Depression and stress are associated with the loss of hippocampal dendritic spines of principal cells, contributing to hippocampal dysfunction. Hippocampal neuroplasticity mechanisms have the potential to mediate rapid mood change. Because pyramidal cell spine synapse remodelling vitally influences hippocampal activity, we hypothesize that major depression are associated with loss of hippocampal spine synapses. Recently, we have confirmed the validity of the new "synaptogenic hypothesis" of depression by demonstrating an inverse correlation between the number of synapses in limbic brain areas and the severity of depressive symptoms, both in animal models and in human beings. It is hypothesized that loss of synapses in depression is, at least partly, caused by prolonged stress and the resultant glutamate excitotoxicity, which could be prevented by antagonizing glutamate release in response to stress. In addition to their anxiolytic, anticonvulsant, muscle-relaxant, and sedative/hypnotic effects, benzodiazepines, such as diazepam, strongly inhibit glutamate release at high, pharmacological doses.

Postpartum depression is a serious clinical problem that affects approximately 10-15% of postpartum women during the six-month period following childbirth. Symptoms of postpartum depression are similar to those of a major depressive episode, exerting a severe impact on family functioning and mother-infant relations in this critical period of life.

To test our theory that remodeling of hippocampal spine synapses also occurs in postpartum depression, we utilized a rat pseudopregnancy model. Ovariectomized CD(SD) rats were subcutaneously implanted with continuous release pellets, providing pregnancy levels of estradiol and progesterone. After 21 days, the hormones were withdrawn and the ensuing week was considered as the postpartum period. "Pregnant" and "postpartum" rats were tested in the learned helplessness paradigm and the number of their hippocampal spine synapses estimated using electron microscopic stereology. Inescapable stress caused a severe loss of spine synapses in "postpartum" animals, while there were no synaptic changes in "pregnant" females. In line with synaptic alterations, performance of "pregnant" rats was significantly better in the active escape test compared to "postpartum" animals.

We can conclude that maintaining pregnancy levels of estradiol and progesterone prevents the synaptic and behavioral effects of inescapable stress, suggesting that the sudden decrease in ovarian hormone levels after childbirth plays a major role in predisposing to postpartum depression.

Our result presents a series of experiments, investigating whether diazepam is able to prevent helplessness and to protect synapses in the learned helplessness (LH) model of depression. Diazepam, when administered intraperitoneally to ovariectomized female CD(SD) rats dose-dependently decreased depressive symptoms in LH and demonstrated synaptoprotective effects in electrophysiological and morphological measurements.

These findings further support the synaptogenic hypothesis of depression and suggest that synaptoprotective treatment is able to antagonize the negative effect of stress on mood, which may be useful in the clinical management of patients with recurrent and/or treatment-resistant depression.

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Occurrence and importance of Aspergilli in agricultural products and clinical sources

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Aspergillus species are filamentous fungi which are widespread on agricultural products in subtropical and tropical areas of the world. Aspergilli are able to produce a range of mycotoxins, which can be harmful to animals or humans, including aflatoxins, ochratoxins, fumonisins and patulin. *Aspergillus flavus* is also an important pathogen of various cultivated plants including maize, cotton and peanut, and cause serious yield losses throughout the world. Since aflatoxin production is favoured by moisture and high temperature, *A. flavus* is able to produce aflatoxins in warmer, tropical and subtropical climates. According to recent studies, climate change accompanied by global warming affects the occurrence of fungi and their mycotoxins in our foods and feeds. A shift has recently been observed in the occurrence of aflatoxin producers in Europe, with consequent aflatoxin contamination in agricultural commodities in several European countries not facing with this problem before (Italy, Serbia, Slovenia, Croatia, Romania, Ukraine). Although aflatoxin contamination of agricultural products is not treated as a serious threat to Hungarian agriculture due to climatic conditions, these observations led us to examine the mycobiota and mycotoxin content of different agricultural products (wheat, maize, chili pepper, nut, etc.) collected from different locations in Hungary and Vojvodina. The surface-sterilized products were placed on selective media, and the isolated fungal strains were identified using morphological and sequence-based methods.

Aspergillus strains are among the most common organisms causing fungal keratitis in tropical and subtropical areas. The main risk factor for the infection is trauma by vegetable matter during agricultural activities. Among *Aspergillus* species, mainly *A. flavus*, *A. terreus*, *A. fumigatus* and *A. niger* have been isolated from fungal keratitis cases. During our study, 52 *Aspergillus* strains isolated from keratitis cases in South India were examined. Based on morphological studies, all isolates were classified to the *A. flavus* species. For the molecular identification, part of the calmodulin gene was amplified and sequenced. As a result, 46 isolates were identified as *A. flavus*, while four as *A. tamarii*, one as *A. terreus* and one was found to belong to the *A. pseudotamarii* species. That was the first case that *A. pseudotamarii*