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عنوان المشروع باللغة العربية - Title of the proposed project in Arabic	العلاج المزمن فيتامين (د) يحمي من الاكسدة الناجم عن بيروكسيد الهيدروجين عن طريق تنظيم أوب من مستقبلات فيتامين د على الخلايا العصبية القشرية الأولية المستزرعة
Title of the proposed project in English	Chronic Vitamin D treatment protects against Hydrogen peroxide induced oxidative stress by Up regulation of Vitamin D receptors on cultured primary cortical neurons
المشرف الرئيس - PI	Samina Hyder Haq
التخصص الدقيق للمشرف الرئيس - Specialty of PI	Tissue culture
المشرف المساعد - Co-PI	Majid Alokail
المدة المتوقعة لإنجاز البحث منذ الحصول على موافقة عمادة الدراسات العليا (بالشهور) - Expected time in month to finish	12 months
Abstract of the proposal (No more than 200 words)	Vitamin D receptor (VDR) and the enzymes involved in bio activation of vitamin D, shown to be expressed in the central nervous system, particularly in areas affected by neurodegenerative disorders [1]. The conflation of in vitro, ex vivo, and animal model data provide compelling

evidence that vitamin D has a crucial role in proliferation, differentiation, neurotrophism, neuroprotection, neurotransmission and neuroplasticity. Vitamin D exerts its biological function not only by influencing cellular processes directly, but also by influencing gene expression through vitamin D response elements [1,2,3]. The effect of Vitamin D during early brain development is very crucial as there is robust evidence from rodent experiments indicating that transient developmental vitamin D (DVD) deficiency is associated with changes in brain structure, neurochemistry, gene and protein expression and behavior. It has been reported that in cultured hippocampal neurons, Treatment with vitamin D3 results in increase in nerve growth factor (NGF) production and neurite outgrowth [4]. In cultured rat mesencephalic neurons, vitamin D3 at low concentrations significantly protects against selective damage to dopaminergic neurons due to a depletion of glutathione [5]. Similarly, pretreatment with vitamin D3 is shown to protect dopaminergic neurons against neurotoxicity by a dopaminergic toxin as well as glutamate [6]. In our earlier studies with cortical neuron culture, we found that treatment of primary cortical neurons with low doses of Vitamin D3 protected the cells from induced oxidative stress by H₂O₂ by increasing antioxidants enzymes such as Reduced glutathione (GSH), Catalase enzyme and reducing lipid peroxidation. However there is a little understanding on the possible mechanism and involvement of the nuclear VDR with DNA binding activity, and up-regulation of VDR gene expression with the chronic Vitamin D3 treatments. The proposed research project will look at the effect of chronic low doses of vitamin D3 treatments on the VDR gene expression to the primary cortical neurons. The aim of this project is to elucidate the fact that chronic treatments of cells with VitaminD3 results in up regulation of VDR which protects the cells against oxidative stress.

Hypothesis of the proposal

Vitamin D has a neuroprotective role as demonstrated by earlier studies in our lab. This research project will unravel the molecular and genetic pathways that control the gene expression of vitamin D receptor.

Specific objectives

To train the graduate students with the technique of primary cell culture .
To establish cell culture and tissue culture facility in Biochemistry department for training of graduate students from all other departments.
To establish for the first time Immunohistochemistry labs and training in our department.

Methodology & Major Techniques to be used

Primary neural cultures will be obtained from 1 week old rat's brain. The cultured neural cells will be set up as follows

1. Control cultures without any treatment
2. Cortical neural cultures treated with 400IU vitaminD3
3. Cortical Neural cultures treated with 0.5mM, H2O2.
4. Cortical Neural cells treated with 0.5mM H2O2 and 400IU vitamin D3.

The cells will be grown in the F12 medium (+glutamine) supplemented with 10%FCS +100units streptomycin +100 units penicillin. For up to 3 days, 7 days, and 10 days in culture. After that the medium will be removed and the cells will be harvested for performing immunohistochemistry and western blotting against the antibody of VDR receptors.

Major techniques involved for this study

1. Immuno-histochemistry
2. western Blotting using odyssey LI-COR machine
3. Setting up primary neural cell culture
4. In Cell vitro Technique using LI-COR
- 5.

Availability of Samples

Yes

Availability of Chemicals

Yes

Availability of Instruments

Yes

Ethical Approval

In the process

Recent References

1. G. C. DeLuca, S. M. Kimball, J. Kolasinski, S. V. Ramagopalan and G. C. Ebers (2013) *Neuropathology and Applied Neurobiology* 39, 458–484.

2. Gezen AD, Dursun E, Yilmazer S, (2013)Vitamin D inquiry in hippocampal neurons: consequences of vitamin D-VDR pathway disruption on calcium channel and the vitamin D requirement. *Neurol Sci* 34(8)1453-8
3. Angub MC, Herman JP, Malluche HH, Koszewski NJ. Evidence of functional vitamin D receptors in rat hippocampus. *Neuroscience*. 2001;104(1):49-56. PubMed PMID: 11311530.
4. Cui X, Gooch H, Groves NJ, Sah P, Burne TH, Eyles DW, McGrath JJ. Vitamin D and the brain: key questions for future research. *J Steroid Biochem Mol Biol*. 2015 Apr;148:305-9. doi: 10.1016/j.jsbmb.2014.11.004. Epub 2014 Nov 6. Review. PubMed PMID: 25448739
5. Shinpo K, Kikuchi S, Sasaki H, Morikawa F, Tashiro K. 2000. Effect of 1, 25-dihydroxyvitamin D₃ on cultured mesencephalic dopaminergic neurons to the combined toxicity caused by L-buthionine sulfoximide and 1-methyl-4-phenylpyridine. *J Neurosci Res* 62:374–
6. Ibi M, Sawada H, Nakanishi M, Kume T, Katsuki H, Kaneko S, Shimohama S, Akaike A. 2001. Protective effects of 1 alpha, 25-(OH)₂D₃ against the neurotoxicity of glutamate and reactive oxygen species in mesencephalic culture. *Neuropharmacol* 40:761–771.