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E V A L U A T I O N

of the dissertation and scientific achievements of Kristine Danielyan Edgar

Honored members of the Specialized Council for Doctoral Theses Defense,

It is indeed a great honor to evaluate the dissertation thesis entitled “PROPHYLACTIC AND THERAPEUTIC EFFECT OF THE NOVEL COMPOUNDS IN THE EXPERIMENTAL STROKE“ submitted to the ARMENENIA INSTITUTE OF MOLECULAR BIOLOGY by Kristine Danielyan Edgar for the Degree of Doctor of Biological Sciences (D.Sc.) in the Field of 03.00.04. “Biochemistry”.

The dissertation summarizes the achievements of the candidate in the field of stroke research in model systems in vitro and in vivo, using both human and rodent neural cell culture systems and rodent models of cerebral ischemia and acute/oxidative brain damage. Stroke is among the top 3 leading causes of severe disability and death worldwide. Effective pharmacological therapy for stroke patients that prevents neuronal death, infarct development or significantly enhances functional recovery and rehabilitation is not available. Current treatment options for ischemic stroke include time-limited application of recombinant tissue plasminogen activator (tPA) within 4.5 h after stroke onset and with a pronounced bleeding risk and potential neurotoxic effects exerted by tPA. More recently, thrombectomy has been established as a highly effective approach in ischemic stroke to mechanically remove thrombi from brain vessels and allow for reperfusion of ischemic brain tissue – with a pronounced enlargement of the treatment window after stroke onset. This may also open a new opportunity for neuroprotective and regenerative strategies which have failed in clinical studies the past but may now be reconsidered for treatment options in the reperfusion phase.
Therefore, work on neuroprotective strategies as proposed in the present dissertation by Kristine Danielyan may open relevant perspectives of neuroprotection for pharmacological treatment of ischemic stroke and other pathological conditions that involve neurodegeneration and progressive structural and functional brain damage.

In particular, ischemic brain damage involves disruption of the blood-brain-barrier (BBB) accelerating cytotoxic brain edema formation thereby contributing to infarct development. Further, oxidative dysregulation and related oxidative cell death is a major hallmark of neurodegeneration after stroke and in many other neurodegenerative disorders. Here, the dissertation by Kristine Danielyan proposes potential therapeutic approaches that include the application of albumin or the xanthin oxidase (XO) inhibitor allopurinol which interfere with mechanisms of brain edema formation and oxidative dysregulation, respectively. In an interesting new approach, the work described here combines these strategies using allopurinol-coated albumin particles for potential application in (model systems of) stroke. The characterization and evaluation of this approach, however, needs further studies in preclinical models of stroke and also for the characterization of the particles. The author claims that these are nanoparticles but reports micrometer sizes and further physicochemical characterization and pharmacological investigations are essential for evaluating this approach.
Further, the neuroprotective potential of another peptide, the vasopressin-analogue PRP-1 is presented in a model of staurosporine-mediated apoptosis. While the neuroprotective effects of PRP-1 are significant, while vasopressin did not exert any protection, the mechanism of action and the relevance of these findings for infarct development after stroke need further evaluation. There is, for example, evidence in the literature that vasopressin receptor signaling is involved in brain damage progression after acute brain injury and vasopressin receptor inhibition was proposed as a potential protective strategy. How this is related to the current findings presented here and how protection against staurosporine is a valid indicator for potential neuroprotective effects in ischemic brain damage, was not discussed and remains unclear. The dissertation also mentions protective effects of PRP-1 in a model of Parkinson's disease (PD), but the data are nor presented and a discussion of the mechanisms of action is not provided.

In addition, beyond the neuroprotective approaches, the work by Kristine Danielyan presents tPA-coated red blood cells (RBC), anti-complement receptor type 1 –tPA (antiCR1/tPA) and anti-PECAMscFv-uPA fusion constructs as potential thrombolytic drugs with high safety profile and low risk for re-bleeding complications. Considering the reported safety issues for tPA when administered too late after ischemic stroke and the established neurotoxic effects of tPA, such approaches provide innovative strategies with enhanced safety in the treatment of thrombo-embolic diseases. However, as discussed by the author, the application of e.g. tPA/RBC is only effective in pretreatment strategies and, therefore, the clinical implication of these innovative strategies may only apply to very isolated indications. In addition, the currently emerging thrombectomy protocols in the treatment of ischemic stroke are apparently very effective and superior to TPA protocols regarding the post-stroke time window, efficacy and safety. Whether the approaches presented here may also support combined surgical and pharmacological approaches is a promising issue that needs further investigation.

A major achievement of Kristine Danielyan's work is the evaluation of the importance of purine and pyrimidine metabolism and according pharmacological approaches in model systems of neuronal death and brain damage relevant to ischemic brain injuries. She investigated the metabolic and neuroprotective capacities of allopurinol and the Phosphoribosyl-Pyrophosphate Synthase 1 (PRPS-1), and also combinations of allopurinol and Vitamine B complex members on XO activity and in model systems of neural cell death in vitro and in vivo. These analyses also included evaluation of human neural cell cultures, which is important for evaluating the relevance of the findings in human cells but also critical regarding ethical issues in science.

Overall, the body of work and the achievements in research on neuronal cell death and neuroprotection in model systems relevant to ischemic stroke presented in the present dissertation are impressive. The work presented here covers major aspects of stroke pathology and related therapeutic intervention ranging from thrombolytic approaches, BBB disruption and brain edema formation to neuroprotection with particular focus on the purine and pyrimidine metabolism that is important for neuroprotection against oxidative dysregulation and brain regeneration.
In particular, the approaches targeting the purine and pyrimidine metabolism and according approaches combining albumin- and allopurinol-mediated protection as well as approaches to improve tPA therapy (safety) are presenting the core of the scientific achievements by Kristine Danielyan, and these are also highly visible and well-received through the according publications, also in highly renowned and leading peer reviewed international journals.
The dissertation and the synopsis represent the major findings of the impressive work by Kristine Danielyan that has been also previously reviewed and approved by international leading experts. Both, the dissertation and the synopsis would have profited from professional language editing, since multiple typographical errors, provided concentration ranges and grammar errors need substantial attendance and unfortunately also distract from reading.

In summary, despite evident formal points in the dissertation and in the synopsis, there is no doubt that the scientific achievements of Kristine Danielyan are exceptional and she has gained international reputation in stroke research with particular expertise in purine/pyrimidine metabolism and according neuroprotective approaches targeting oxidative dysregulation and regenerative strategies as well as in novel approaches for tPA formulations with some therapeutic potential in selected prophylactic antithrombotic therapy. Therefore, I fully support her nomination and recommend acceptance of her application for the degree of Doctor of Biological Sciences in the Field of “Biochemistry”.

Sincerely,