Role of MAP kinases and PI-3-kinase/Akt pathway in the regulation of retinal degeneration

Ph.D. thesis

Author: DR. MESTER LÁSZLÓ

Department of Biochemistry and Medical Chemistry

University of Pécs Medical School

Pécs, Hungary

2010

1. INTRODUCTION

Ischemia and oxidative stress alone or as a part of ischemia can be found in the pathogenesis of several disease affecting large populations in developed countries. Such diseases are particularly the cardiovascular, neurovascular diseases and neurodegenerative disorders. From the aspect of ophthalmology, various ocular and systemic diseases that lead to visual impairment or blindness (e.g., central retinal artery occlusion, ocular ischemic syndrome, diabetic retinopathy, hypertension, glaucoma and AMD) are accompanied by retinal ischemia (Uckermann et al. 2005, Osborne et al 2004, Feigl 2009). Furthermore, the light absorption of the retina generates increased formation of oxidative/nitrosative agents, which may cause retinal injury as it can be observed in a vision-threathening retinal disease, the age-related macular degeneration (AMD) (Liang et al 2003, Winkler et al 1999, Cai et al 2000, Jarret et al 2008).

It is noticeable that ischemia and oxidative stress both can be found in the pathogenesis of diseases leading the “toplist” of blindness in the developed world (AMD, glaucoma, DRP). Hence, it is of utmost importance to understand the events involved in retinal injury caused by ischemia and/or oxidative stress, both from the pathological and the potential therapeutic point-of-view.

Retina: Embriologically the retina belongs to the central nervous system. Anatomically it is a very delicate, and transparent membrane. The main function of the retina is transforming the light-stimuli from the outside world into a nerve impulse that reaches the brain via the optic nerve. The retina is loosely attached to the choroid via the retinal pigment epithelium (RPE), that consists of a monolayer of hexagonal cells. The main functions of the RPE are: vitamin A metabolism, maintenance of the outer blood-retinal barrier, phagocytosis of the photoreceptor outer segments, absorption of light, heat exchange, formation of the basal lamina, production of the muccopolysaccharide matrix surrounding the outer segments, and active transport of materials in and out of photoreceptors.

Ischemia: Ischemia refers to a pathological situation involving an inadequacy (not necessarily a complete lack of) blood flow to a tissue, with failure to meet cellular energy demands. The ischemic injury reflects the effect of a self-reinforcing destructive cascade called “ischemic cascade”, which is an extremely complex (not completely understood)

succession or cascade of interrelated pathological changes and biochemical responses at the cellular and molecular level initiated by energy failure.

Oxidative stress: Oxidative stress represents an imbalance between the production of reactive oxigen species (ROS) and biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. The excessive formation of ROS leads to lipid peroxidation, protein oxidation and DNA. All of these effects in connection with the mitochondrial damage results in cell death, hence ROS has an important role in the pathogenesis of diseases, including neurodegenerative and cardiovascular diseases.

PARP (poly(ADP-ribose) polymerase)**:** The abundant nuclear enzyme PARP-1 is activated by single- and double- strand breaks of DNA. For many decades, PARP was mainly viewed as an enzyme primarily involved in DNA repair and maintenance of genomic stability. However, over the last decade, an additional role of PARP has been identified in the sequale of oxidative and/or nitrosative stress. The PARP-1 is involved in the development of diseases associated with oxidative stress with dual mechanism: 1. excessive activation of the enzyme results in cell death caused by energy deficiency, 2. the enzyme is involved in the regulation the NFkB-dependent transcription of inflammatory mediators. Therefore, in diseases where necrosis dominates (stroke, myocardial infarction, arteria centralis retinae occlusion) the former, while in the inflammatory type of diseases not accompanied by massive cell death (colitis, diabetes, uveitis, arthritis) rather the latter mechanism dominates.

PACAP: PACAP belongs to the vasoactive intestinal peptide (VIP)/secretin/glucagon family. The biological actions of PACAP are very diverse. Among others, the neuropeptide influences reproductive functions, circadian rhythm, thermoregulation, feeding, depression, memory, urinary reflexes, inflammatory reactions, and development. PACAP has well-established neurotrophic and neuroprotective functions as well.

2. STUDY OBJECTIVES

Although involvement of PARP activation in various ischemia models has been thoroughly studied, only circumstantial evidences are available for the role of PARP activation in chronic hypoperfusion-induced neurodegenerative processes. Therefore, the aim of the present study was the following :

- to demonstrate the activation of PARP as a major regulator of cell death in a chronic hypoperfusion-induced retinal degeneration model in rat (bilateral common carotid occlusion induced retinal degeneration)

- to evaluate the effect of PARP inhibition (by HO3089) in this model by assessing chronic hypoperfusion-induced morphological changes

- to determine the activation state of critical kinase cascades, such as MAP kinases and PI-3K-Akt in hypoperfusion-induced retinal degeneration

In spite of the numerous studies showing the protective effects of PACAP in the retina, no data are currently available on the potential protective effect of PACAP against oxidative stress in pigment epithelial cells. Therefore, it seemed reasonable to study whether PACAP is able to increase cell survival in oxidative stress-induced apoptosis of human pigment epithelial cells. The aim of present study was the following:

- to elucidate the effect of PACAP on cultured human pigment epithelial cells (ARPE-19 cells) in oxidative stress

- to detect the effect of PACAP on apoptosis and necrosis on cultured ARPE-19 cells by Annexin V and propidium iodide staining

- to detect the effect of PACAP on mitochondrial depolarization occurring in apoptosis by using the JC-1 assay for flow cytometry

3. CONCLUSION

The activation status of PARP in BCCAO model

- We provided evidence for establishing PARP activation as a major regulator of the cell death process in chronic hypoperfusion-induced neurodegeneration. Activation of PARP in the retina was revealed by assessing poly-ADP-ribosylation of target proteins. Treatment of the eye with the PARP inhibitor -HO3089- attenuated the BCCAO-induced self-poly-ADP-ribosylation of PARP.

The effect of PARP inhibitor –HO3089- on retinal morphology in BCCAO model

- We provide histological evidence for the retinoprotective effect of PARP inhibition. Intravitreal PARP inhibition -HO3089- treatment following BCCAO led to a nearly intact appearance of the retinal layers.This is well supported by the morphometric measurements.

The involvement of different cell signaling pathways in the mechanism of PARP-inhibition-induced neuroprotection in this model.

- We determined that activation of PI-3K-Akt and ERK1/2 was cytoprotective, and inhibition of JNK and p38 MAPK cytotoxic signaling pathways were very likely involved in the mechanism of PARP-inhibition-induced neuroprotection in this model.

In summary, based on these results PARP inhibition may represent a molecular target in the clinical management of ocular ischemic syndrome, and in a broader sense, chronic hypoperfusion-induced neurodegenerative diseases.

Effect of PACAP on cell viability of human pigment epithelial cells

- We showed that PACAP treatment diminished the effect of cell death caused by H_2O_2 treatment in retinal human pigment epithelial cell line. Furthermore, the effect of PACAP1-38 could be blocked by PACAP6-38 (inhibitor of PACAP1-38) co-application

Concentration-dependency of PACAP

- We found that the protective effect of PACAP was concentration dependent. From the concentration range of 1pM to 1µM, the best result was achived by 100 nM PACAP1-38 treatment.

Effect of PACAP in cell death

- PACAP administration led to a significant increase in the percentage of living cells and a reproducible decrease in the rate of apoptosis in cells treated with H_2O_2.

Effect of PACAP on mitochondrial depolarization

- An increase of apoptotic cell number (mitochondrial depolarization) was observed in the H_2O_2-treated group with a lower number of living cells. PACAP administration led to a significant increase in the percentage of living cells and a decrease in the percentage of apoptotic cells exposed to H_2O_2.

In summary, our present results show that PACAP has antiapoptotic effects against oxidative stress-induced cell death in retinal human pigment epithelial cells, providing an additional piece of evidence for the retinoprotective effects of PACAP. Thus PACAP may take part in future clinically effective treatments of retinal diseases caused by oxidative stress.

4. PRACTICAL MEANING

There are extremely complex molecular mechanisms in intracellular signaling that we still do not know. Thus the identification and the elucidation of the role of these pathways are particularly important since it leads to identification of new drug targets and this can be the first step in the development of new therapeutic agents. On the other hand, the “deeper” exploration of the mode of action of new and existing drugs give the clinician the chance to treat the patient more effectively.

5. ACKNOWLEDGEMENTS

I would like to thank to my program leader Prof. Dr Balázs Sümegi for having given the possibility to work at the Department of Biochemistry and giving me support. I am grateful to Dr Dóra Reglődi and Prof. Dr Ferenc Gallyas Jr. for their tutorial work.
I would like to thank the following people who worked with me during the past 3 years and helped my work not only with giving excellent professional advice, but with maintaining friendly atmosphere:

Dr Krisztina Kovács, Alíz Szabó, Enikő Hocsák, Dr Péter Kiss, Krisztina Szabadfi, Tamás Atlasz from University of Pécs and Prof. Dr Bálint Kovács, Dr Balázs Kovács and all from the Department of Ophthalmolgy of the Kaposi Mór County Hospital.

This work is based on the following articles:

L Mester, A Szabo, T Atlasz, K Szabadfi, D Reglodi, P Kiss, B Racz, A Tamas, F Gallyas Jr, B Sumegi, E Hocsak, R Gabriel, K Kovacs

Protection Against Chronic Hypoperfusion-Induced Retinal Neurodegeneration by PARP Inhibition via Activation of PI-3 kinase Akt Pathway and Supression of JNK and p38 MAP kinases

Neurotox Res (2009) 16:68-76

IF: 2,439

L Mester, K Kovacs, B Racz, I Solti, T Atlasz, K Szabadfi, A Tamas, D Reglodi

Pituitary Adenylate Cyclase-Activating Polypeptide is Protective Against Oxidative Stress in Human Retinal Pigment Epithelial Cells

J Mol Neurosci (2010) (In Press, PMID: 20645022)

IF 2,720

K Szabadfi , **L Mester** , D Reglodi , P Kiss , N Babai , B Racz , K Kovacs , A Szabo , A Tamas, R Gabriel, T Atlasz

Novel Neuroprotective Strategies in Ischemic Retinal Lesions

Int. J. Mol. Sci. (2010), Feb 3;11(2):544-61

IF: 1,387

Further publications:

V Mester, F Kuhn, **L Mester**

Epiretinal membranes: current management concepts

Expert Review of Ophthalmology (2007), Feb Vol. 2, No.1, Pages 131-141

E Hocsak, B Racz, A Szabo, **L Mester**, E Rapolti, Sz Javor, Sz Bellyei, F Gallyas Jr., B Sumegi, A Szigeti

TIP47 protects mitochondrial membrane integrity and inhibits oxidative-stress-induced cell death

FEBS Letters (2010) Jul 2;584(13):2953-60.

IF 3,541

V Mester, F Kuhn, **L Mester**

Éles tárgyak által okozott szemsérülések prognózisa

Kovács Bálint professzor a Pécsi Szemklinika Igazgatója 1988-2008, Emlékkönyv

2008, Pages 103-110