ORAL PRESENTATION • DISCOVERY SCIENCE

[OPS1]

ACUTE EXPOSURE OF RAT RETINAS TO LOW DOSE ENDOTHELIN-1 RESULTS IN SUPPRESSION OF THE CLASSICAL RAAS AXIS AND ACTIVATION OF THE ALTERNATIVE AXIS

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ET1 is the most potent vasoconstrictive peptide and there is evidence suggesting its important role in retinal degeneration. It is hypothesised that ET1-induced vascular changes may lead to ischemia and excitotoxicity, important pathophysiological factors in glaucoma. Remarkably, almost all components of renin-angiotensin-aldosterone system (RAAS) are expressed in retina and may play a crucial role in the fate of the ischemic retinal tissue; however, the effect of ET1 on RAAS expression remains unclear. The objective of current study was to investigate effect of ET1 at a dose of 200 pmol on the protein expression of glutamate and RAAS components in rat retinas.

Sprague Dawley Rats of either sex (150-300 g) were randomly allocated into two groups of 54 animals each: control group received PBS while the other group received ET1, intravitreally. Seven days after injections, the rats were sacrificed, and their retinas were harvested. The retinal samples were homogenised and subjected to ELISA to estimate the angiotensinogen, renin, angiotensin I (Ang I), angiotensin-converting enzyme (ACE), angiotensin II (Ang II), angiotensin type 1 receptor (AT1R), angiotensin-converting enzyme 2 (ACE2), angiotensin 1-7 (Ang 1-7), aldosterone and glutamate expression.

The expression of Ang I, Ang II, glutamate and aldosterone were significantly decreased in ET1-exposed retinas compared to the control group, whereas the expression of ACE2, Ang 1-7 and AT1R were significantly upregulated. It suggests that retinal ischemia caused by ET1 at a dose of 200 pmol stimulates a response by upregulating the protective ACE2/Ang 1-7/Mas receptor and downregulating the classical ACE/Ang II/AT1R pathway.

Acute exposure of rat retinas to ET1 at a dose of 200 pmol results in activation of endogenous protective mechanisms via suppression of the classical RAAS axis, formed by ACE, Ang II, and AT1R, and activation of the alternative axis comprising ACE2 and Ang 1-7.

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[OPS2]

ROLE OF RENIN-ANGIOTENSIN ALDOSTERONE SYSTEM IN EXCITOTOXIC RETINAL DAMAGE IN RATS

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Glaucoma is the second leading cause of blindness globally. Retinal ganglion cell loss is the hallmark of glaucoma. Current treatment strategies for glaucoma focus on decreasing intraocular pressure. Despite normal intraocular pressure, retinal ganglion cell loss can continue. Ocular Renin-Angiotensin Aldosterone-System (RAAS) could be a potential therapeutic target for neuroprotection. N-methyl-d-Aspartate (NMDA) receptors are the main mediators of the excitotoxic effects of glutamate. This project aimed to investigate whether retinal RAAS expression was altered by excitotoxic injury. The objective of the study was to determine the role of RAAS in excitotoxic retinal damage by measuring the effect of NMDA on the expression of retinal renin, angiotensin II – 1 receptor (AT1R), angiotensinogen, angiotensin II (Ang II), angiotensin-converting enzyme (ACE), ACE-2, Ang-(1-7) and aldosterone, using enzyme-linked immunosorbent assay.

Adult Sprague Dawley rats weighing between 150g-300g were randomly divided into 2 groups: negative control (group 1, n=54) and NMDA (group 2, n=54). The rats were injected intravitreally with phosphate buffer saline (PBS) for control and NMDA for the second group. After seven days, the rats were sacrificed, their eyes were enucleated, and retinas were extracted and subjected to ELISA for the measurement of the above parameters.

Exposure to NMDA enhanced the expression of classical RAAS and suppressed the alternate RAAS in rat retina as reflected by significantly greater expression of angiotensinogen ($p<0.001$), renin ($p<0.05$), ACE ($p<0.001$), Ang II ($p<0.001$), AT1R ($p<0.001$) and significantly lower expression of ACE-2 ($p<0.05$) and angiotensin 1-7 ($p<0.001$).

The classic pathway of RAAS is activated by NMDA in rat retinas while the alternate pathway is suppressed, which has a protective role. This may contribute to the pathogenesis of glaucoma and could be considered an important therapeutic target. Further studies can be done regarding the role of drugs affecting the RAAS system as neuroprotective agents in glaucoma.

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[OPS3]

IN VITRO ANTIOXIDATIVE, ANTI-CHOLINESTERASE, ANTI-α-GLUCOSIDASE AND ALBUMIN DENATURATION INHIBITORY ACTIVITIES OF GELATIN-PHENOLIC ACID CONJUGATES

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Oxidative stress is a culprit of many age-related diseases (ARD), including Alzheimer's disease, diabetes mellitus, and inflammation. The objective of the study was to prepare conjugates of gelatin-2,3-dihydroxybenzoic acid (Ge-2,3-DHBA) and gelatin-2,5-dihydroxybenzoic acid (Ge-2,5-DHBA)) to investigate their antioxidant, anti-cholinesterase, anti-α-glucosidase and albumin denaturation inhibitory activities.

The conjugates were synthesised by the reaction between Ge and 2,3-DHBA and between Ge and 2,5-DHBA, through a simple one-step reaction in the presence of water-soluble redox initiators (H_2O_2 and ascorbic acid) via radical mediated reaction at room temperature. The antioxidant activities of Ge-2,3-DHBA and Ge-2,5-DHBA were measure with 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay, superoxide assay, hydrogen peroxide assay and hydroxyl assay. The enzymatic activities of Ge-2,3-DHBA and Ge-2,5-DHBA were determined using the anticholinesterase inhibitor screening kit, anti-α-glucosidase inhibitor screening kit and albumin denaturation inhibitor screening kit.

Ge-2,3-DHBA had significantly higher DPPH and hydroxyl radical scavenging activity than Ge-2,5-DHBA ($p<0.0001$), while Ge-2,5-DHBA had significantly higher superoxide and hydrogen peroxide radicals scavenging activity than Ge-2,3-DHBA ($p<0.001$ and $p<0.05$, respectively). Ge-2,3-DHBA showed stronger inhibition towards acetylcholinesterase and α-glucosidase enzyme, as well as albumin denaturation, at all concentrations, in comparison to Ge-2,5-DHBA, but no significant differences were found between these two conjugates at all concentrations ($p>0.05$).

Ge-2,3-DHBA and Ge-2,5-DHBA conjugates synthesised exhibited antioxidant activities, anti-cholinesterase, anti-α-glucosidase and albumin denaturation inhibitory activities. These results indicated that Ge-2,3-DHBA and Ge-2,5-DHBA could be beneficial in preventing oxidative stress and treating ARD. However, further in-vitro and in-vivo studies should be conducted to determine their stability and pharmacokinetics for future drug development.