Proceedings of the 2nd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/15/20240476

Extracellular EDPs at high concentrations participate in neuronal cytoskeleton assembly and AD etiology

Zihan Weng

Shanghai American School Pudong Campus, Shanghai, China

zihan01pd2024@saschina.org

Abstract. Elastin in the Extracellular Matrix provides tissues with flexibility and degrades over time to release biologically active Elastin-Derived Peptides (EDPs). EDPs have recently been discovered to potentially mediate pathological conditions of Alzheimer's Disease (AD). However, few studies have shown the regulation of EDPs on the neuronal function during ADprogression. Firstly, a meta-analysis on 11 related studies was conducted to pre-verify the hypothesis and to reduce experimental bias. After that, experiments on a mouse hippocampal cell-line (HT22) were set to test for both cell vitality and morphology after exposure to EDPs at different concentrations (0.0001 μM-50 μM) and durations (0-48 h). Cell vitality was assessed quantitatively using a cell counting kit and cell morphology assessed qualitatively using phalloidin staining on actin filaments. A rescue experiment was also set to discover potential ways of mitigating the effects of EDPs on cytoskeleton assembly. Data here showed statistically significant effects of EDPs on impacting cell vitality negatively at higher concentrations (>0.1 μM) and longer durations (48 h). Cell morphology after EDPs incubation was also impacted with less F-actin stress fibers, which are essential for effective neuronal transmissions. Lastly, rescue experiments using galactose was found to considerably reduce the effects of EDPs on cell morphology. It was concluded that EDPs does negatively impact cell vitality and morphology of neurons. Given the cytoskeleton structures and assembly are important for neuronal function, the data indicated a pathological role of EDPs on the progression of AD.

Keywords: Elastin-Derived Peptides, Dementia, Neuron, Cytoskeleton.

1. Introduction

The Extracellular Matrix (ECM), "scaffoldings" for tissues and organs occupying spaces between cells, are home to various structural and functional proteins and proteoglycans [1]. One important protein present in the ECM is elastin, primarily granting tissues and organs with resilience and elasticity. Elastin are large elastic fibers composed from its cross-lined monomer precursor tropoelastin (TE), thus granting it with extreme flexibility and durability (with half-lives over 70 years and roughly 1000 times more flexible than collagen). However, elastin slowly degrades overtime due to proteolytic activity to release bioactive hexapeptides named elastin-derived peptides (EDPs) [2]. The release of EDPs within the brain are especially critical for one's health, and an increase in EDPs levels within the cerebral spinal fluid of patients with Alzheimer's Disease (AD) or strokes has been shown by clinical research [3]. Animal experiments also confirmed phenotypes in AD model mice treated with elastin-like peptides

DOI: 10.54254/2753-8818/15/20240476

(ELPs), suggesting a close relationship between accumulated EDPs and the promotion of the disease, yet the effects of these released EDPs on AD etiology remain elusive.

AD, the most common type of dementia, is a condition characterized by the constant loss of intellectual ability. The symptoms of AD are usually progressive, starting with memory loss, and eventually evolving into the loss of ability to communicate or even response to the environment on a whole. Treatment for AD is thus extremely difficult, as the etiologies of AD is complex and the mechanistic elucidation of AD pathogenesis remains inconclusive [4]. There is therefore an urgent need for the discoveries of novel targets or biomarkers for the progression of AD, as the clinical diagnosis of the disease will then be more structured.

In terms of age-related AD pathology and the increased EDPs level in old people, the effects of EDPs on AD however is still unclear. There is a clear correlation between rising levels of EDPs and the development of AD in previous studies, suggesting it as a potential cause for AD. Elastin-like Peptides (ELPs), artificially produced EDPs has also been shown to cause the up-regulation of Amyloid-beta (Aβ) proteins in mouse models [5]. Unfortunately, most published results provide evidence that EDPs is involved in the survival, healing or inflammation of astrocytes and microglia—with none being conducted on neurons, the basis of all mental activities.

Accordingly, this research therefore aims to verify the how EDPs affect neuronal activity/morphology and promote the advancement of AD. If direct influences are observed through the study, a new biomarker that steadily rises throughout the progression of AD will be observed and may be used in future diagnosis of the disease.

2. Materials and Methods

2.1. Meta Analysis

2.1.1. Search Strategies. Prior to lab experimentation, meta-analysis evaluating the relationship between EDPs and AD/Dementia/Stroke were conducted to limit bias and pre-verify a correlation. All studies were screened within the database PubMed until January 10, 2023. No limitations on publication date were placed upon the initial screening.

The search was screened using combination of Elastin Peptide and either AD, Dementia, or stroke. Searches with these key words yielded 52 potential studies.

2.1.2. Inclusion and Exclusion Criteria. The 52 potential studies were then screened to include or exclude based on multiple metrics. Exclusion criteria include: 1) Study type: Whether or not the study were experimental or not (Not reviews); 2) Studies non-related to AD/Dementia/Stroke; 3) Studies non-related to EDPs.

Out of the 52 potential studies found, 11 studies were kept to be carried out for reviews (Figure 2). These 11 studies include 7 Human/Clinical studies and 4 Animal/Cell studies.

DOI: 10.54254/2753-8818/15/20240476

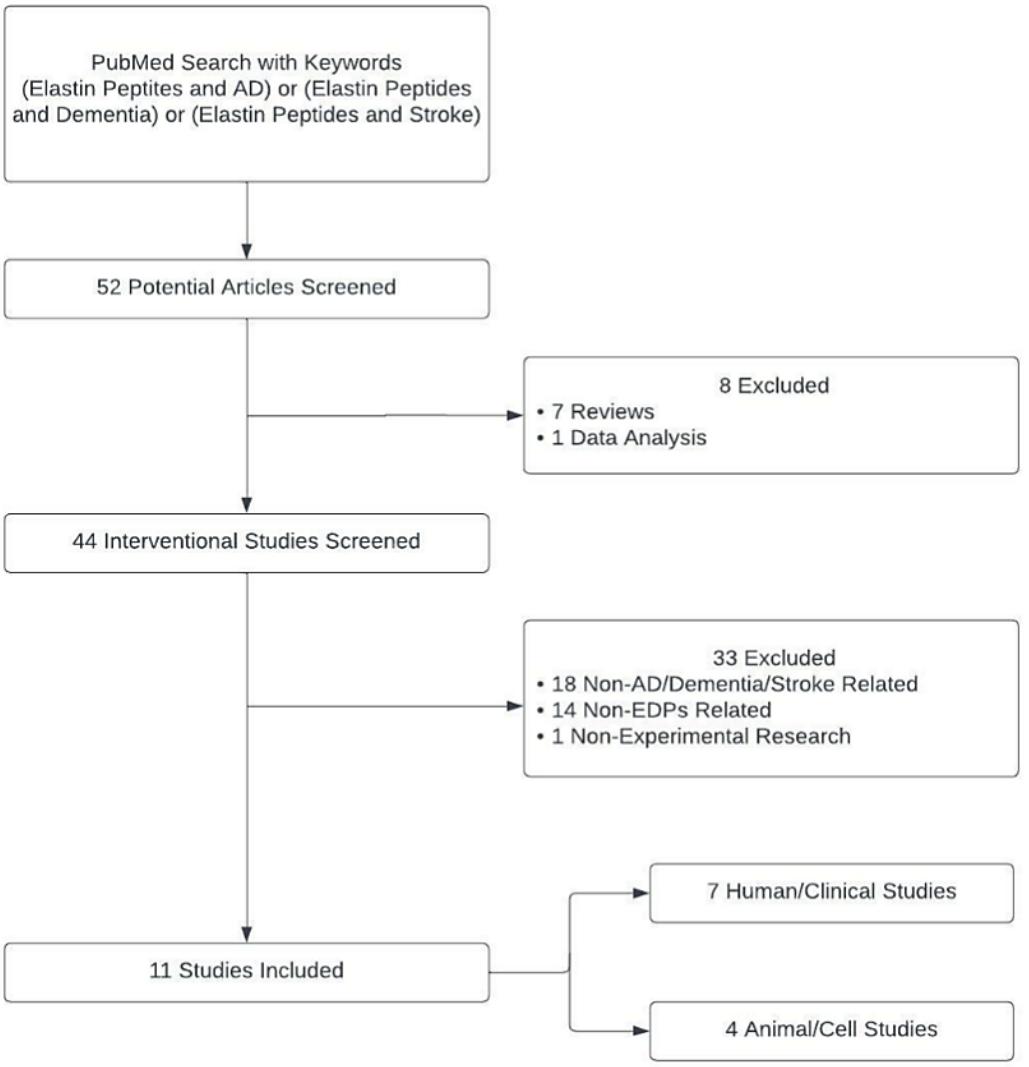

Figure 1. PRISMA Diagram for screening process of Meta-analysis. All sources are taken from the online database *Pubmed*, and screened based on study type and relevance. 11 studies were taken, including 7 human/clinical studies and 4 animal/cell studies.

2.1.3. Data Collection. Data for both the control group and experimental groups were collected from the studies and organized into a document. Data extracted include: 1) Basic Information: Name of author, article title, publication year etc. ; 2) Subject data: Population, age of patients (clinical studies), number/group etc. 3) Study type and methods: Experimental methods; 4) Outcomes: affect of EDPs on AD/Dementia/Stroke.

2.1.4. Risk of Bias. Risk and Bias assessments using the risk bias assessment tool in Review Manager 5.4 were also carried on all 11 studies in 7 different areas. Namely: 1) Selection Bias in random sequence generation; 2) Selection Bias in allocation; 3) Performance Bias; 4) Detection Bias; 5) Attrition Bias; 6) Reporting Bias; 7) Other Bias. Answers to these biases are responded as either Low risk of bias, High risk of bias, or Unclear risk of bias.

2.1.5. Statistical Analysis. After confirming the integrity of the studies, the clinical/experimental data were then analyzed quantitatively with Review Manager 5.4, a software designed to facilitate statistical

DOI: 10.54254/2753-8818/15/20240476

analysis and literature review. The software autogenerates forest plots based on entered data (mean value, standard deviation, # of trials) of patients and control samples and suggests statistical significance.

2.2. Cell Culture

HT-22 is a mouse hippocampal cell-line originally derived from immortalized mouse neuron tissues. It is extremely sensitive to glutamate signaling and especially to the associated cytotoxicity. These types of glutamate-induced cytotoxicity are also commonly related to many mental disorders including AD, Parkinson's Disease etc. The cell-line is chosen for this specific study as it resembles reactions of a human neuron cell in a micro-environment.

To prepare for experimentation, HT-22 cells were cultured in Dulbecco's modified Eagle's medium with high glucose (DMEM) including 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cultures are kept in Petri dish (d = 6cm) during the culturing process. Assessment of cell viability and morphology is subsequently conducted as follows.

2.3. Cell Viability Assay

Cell viability was measured using Cell Counting Kit 8 (CCK-8) following all manufacturer's instructions. CCK-8 provides a convenient way to assess cell viability. The kit includes a soluble tetrazolium salt, a chemical which is reduced by cellular dehydrogenases in living cells only into an orange-colored formazan. The concentration of this orange formazan is therefore directly proportional to the concentration of living cells present.

The HT-22 cells were first divided into two groups— EDPs and Control, and were placed in 96-well plates. After the cells matured, EDPs with formula $(VGVAPG)_3$ were added at different concentrations (0.0001, 0.001, 0.01, 0.1, 1, 10, 50 μM) to the cells. An innocuous peptide of the same molar mass with formula $(VVGPGA)_3$ was then added to the control group.

10 μL of CCK-8 solution was added to each well after 24 and 48 hours. The absorbance were then logged using a micro-plate reader at 450nm. All experiments were performed in triplicate.

2.4. Cell Morphology and Rescue Experiment

For cell morphology, HT-22 cells are plated on poly-L-lysine-coated 12 mm coverslips overnight followed by treatment with a suitable concentration and duration of 10 μM and 48 h for both the EDPs and control groups. The yielded result will be presented as an image using phalloidin staining.

Rescue experiments are also conducted with chondroitin sulfate and galactose based upon a recent study suggesting their ability to reduce EDPs signaling. For the rescue experiment EDPs and chondroitin sulfate/galactose was added together to cultured cells at 10 μM and 20 μM respectively, with results being taken after 48 hours also through phalloidin staining.

Phalloidin images for cell morphology in both the peptide treatment and rescue experiments are assessed qualitatively. The general standard for the functionality of a cell however is based upon the amount of stress fibers present as well as how stretched out the dendrites of the cells are.

2.5. Western Blotting

The above tests all aim to identify the effects of EDPs on neurons through a macro lens. Both cell vitality and morphology are results of small chemical reactions happening on a micro scale. Consequently, in order to assess this small-scale change causing overall stress in cytoskeleton arrangement, processes included with f-actin organization has to be investigated. Since the hypothesis suggests that overall cytoskeleton structures are damaged, the number of functional actin filaments also decreases in EDPs regulated cells. This distortion in shape hence most likely arises from excess cleansing and cutting of existing filaments, or an over-activation of cofilin, a type of actin-binding proteins associated with actin modification and overall cell shape. The phosphorylation levels of cofilin proteins within neurons therefore determine the state of actin filaments and the subsequent shape of neurons. An over-activation will be seen as an overall decrease in phosphorylation levels in cofilin proteins, which can then be measured using western blotting.

DOI: 10.54254/2753-8818/15/20240476

Western blotting is a laboratory technique used for detecting specific proteins. Through attaching antibodies to cofilin proteins and the phosphate groups, the relative ratio of phosphorylated cofilin can be observed. Comparison between EDPs and control groups will then confirm whether or not an up-regulation of cofilin activation is present.

2.6. Statistical Analysis

Quantitative data from CCK-8 on cell vitality are analyzed using Prism 7.0 by a two-tailed t-test between the control and EDPs groups on a 95% confidence interval. Phalloidin staining data of control, EDPs, and rescue groups are assessed qualitatively based on cell morphology. Western blotting on cofilin proteins will also be assessed qualitatively based on visible color representing concentration.

3. Results

3.1. Strong Correlation Exists between Rising EDPs Levels and the Progression of AD

Meta analysis shows statistically significant relationships between rising EDPs levels and the formation of AD symptoms. According to the autogenerated forest plot (Fig. 2) based on entered data shown below, the overall effects of EDPs (shown with the diamond shape) doesn't rest upon the middle, or null result line (Fig. 2). This suggests that considering all studies and possible deviations within data, correlation most likely exists ($P<0.00001$) between an increase in EDPs levels and the development of AD.

Assessment on the overall credibility of the sources was also evaluated through the form of a risk and bias chart (Fig. 3). As shown below, the risk of bias for all categories are relatively low, with only a few articles missing specific information in the selective or blinding process. This suggests that the correlation seen in the forest plot is accurate enough for the lab portion of the research to continue.

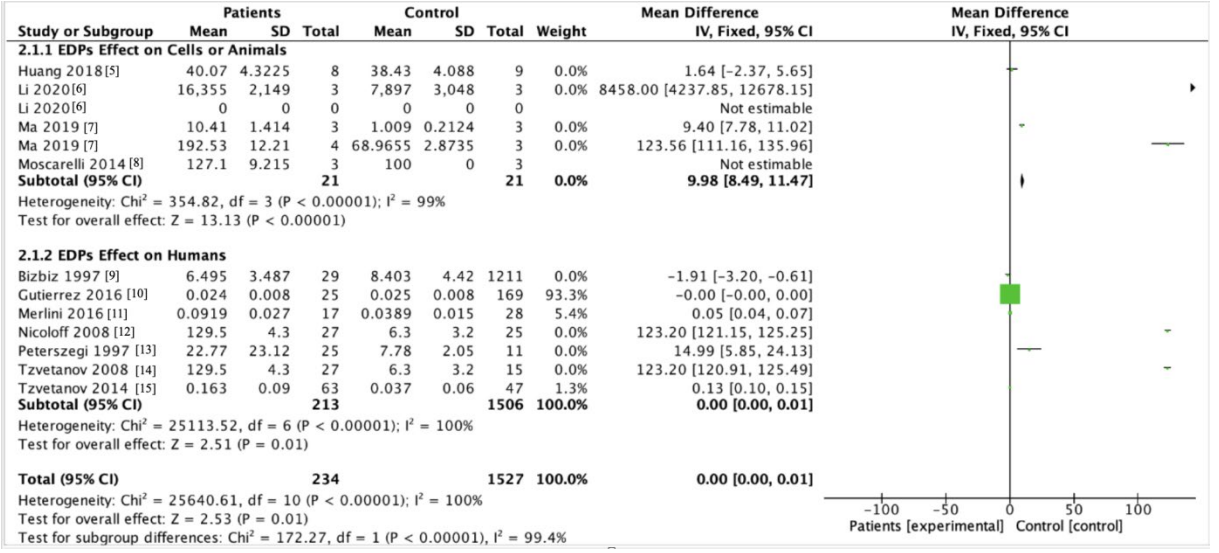

Figure 2. Forest Plot shows comparisons between patients and control groups of each study on the difference in the independent variable (EDPs levels) and dependent variable (AD, dementia, Stroke). Data of each study is shown by the green boxes, with its size proportional to patient/control size of each study. A 95% confidence interval is shown by the lines around each study. The overall correlation, shown by the diamond shape, is a combination of all studies. Since the diamond doesn't rest upon the null result line in the middle, the correlations between the independent and dependent variable are statically significant on a 95% confidence interval. The exact P value is less than 0.00001, suggesting strong statistical significance.

DOI: 10.54254/2753-8818/15/20240476

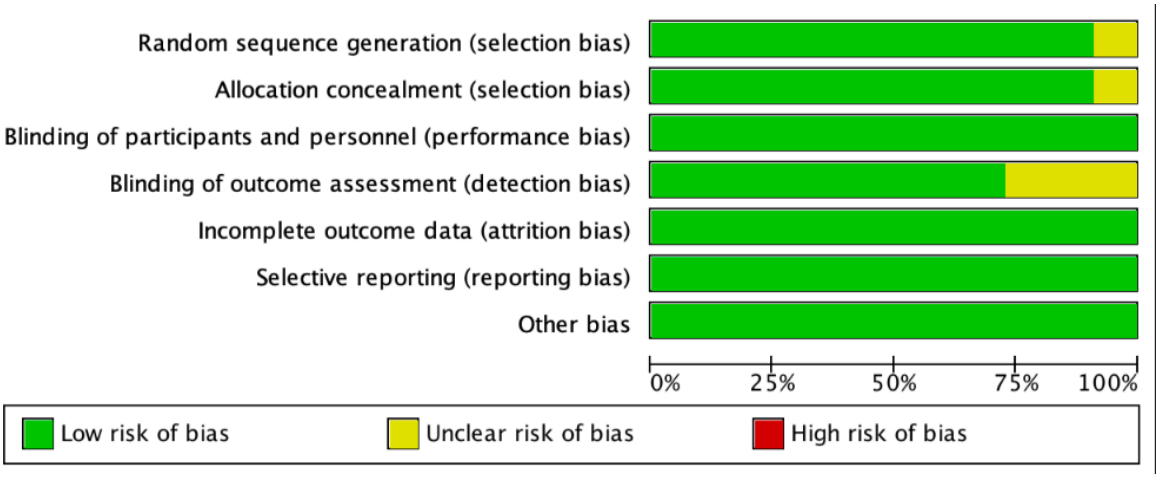

Figure 3. Risk and Bias Chart. Objective analysis on the overall bias of each article for the shown categories, with green meaning low risk of bias, yellow meaning unclear risk of bias, and red meaning high risk of bias. Shown percentage is taken of the assessed 11 articles for the meta-analysis.

3.2. EDPs at High Concentration Negatively Influence Cell Vitality

CCK-8 data suggests that EDPs negatively influence cell vitality at higher concentrations and long durations of exposure. As shown by Fig. 4, though a slightly downward sloping trend can be seen, EDPs at all concentrations at 24 h had insignificant impact on cell vitality. EDPs at concentrations lower than 0.1 μM at 48 h were also insignificant. However, a sudden drop with cell vitality exists for concentrations 0.1 μM and above at 48 h. Four sets of data points were found to be statistically significant, with an increasingly downwards facing trend for EDPs at higher concentrations.

This data demonstrates that EDPs may pose cytotoxic to neurons, with greater concentration of EDPs and longer durations of exposure taking greater effects on the overall survival of cells.

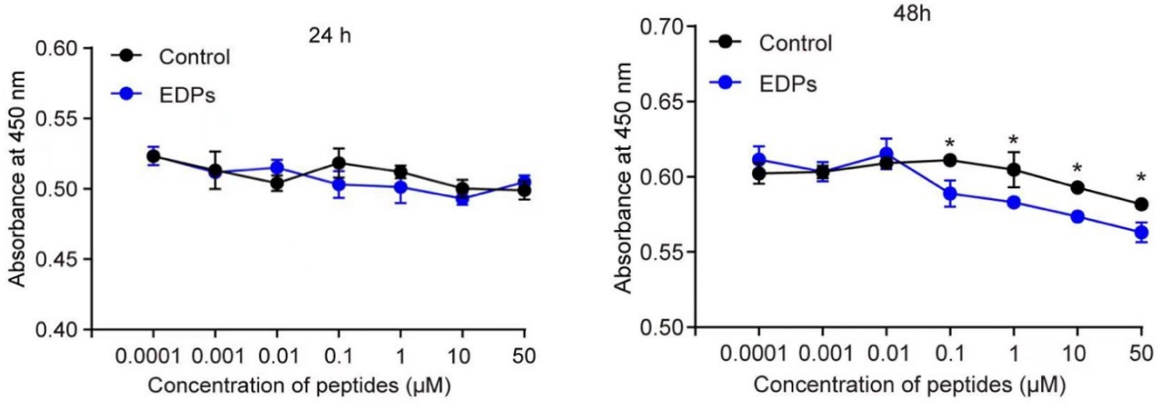

Figure 4. Cell vitality data using cell counting kit 8 (CCK-8). Absorbance at 450 nm for both control and EDPs groups at each concentration is collected for both 24h and 48h. The black line indicates absorbance of control groups with 2 standard deviations of each data set. The blue line indicates absorbance of EDPs groups with 2 standard deviations of each data set. Statistical significance is tested on a 95% confidence interval. For all concentrations at 24h, the difference in level is insignificant. All concentrations below 0.1 μM is also insignificant. However, statistically significant data is collected between control and EDPs groups at concentrations 0.1 μM $\leqslant$ at 48h.

DOI: 10.54254/2753-8818/15/20240476

3.3. EDPs Distort Cell Morphology and Cytoskeleton Assembly

Assessment of phalloidin staining of cells shows a clear influence EDPs has on cytoskeleton structure and cell morphology. As shown from the images below (Fig. 5), actin filaments (stained red) in the control groups are much more extended with clearer dendrites compared to the EDPs groups. HT-22 cell-lines exposed to EDPs instead are shown to be rounder, suggesting the cytoskeleton losing its function and being unable to support the shape needed for effective neuronal transmissions. In addition, the number of actin stress fibers are much more condescended and appear more frequently in the control groups.

Evaluations of the images therefore clearly suggest the negative effects EDPs has on cell structures. Consequently, further to a loss in overall numbers, EDPs also influences the functionality in living cells through influencing cytoskeleton assembly within neuron-like cells.

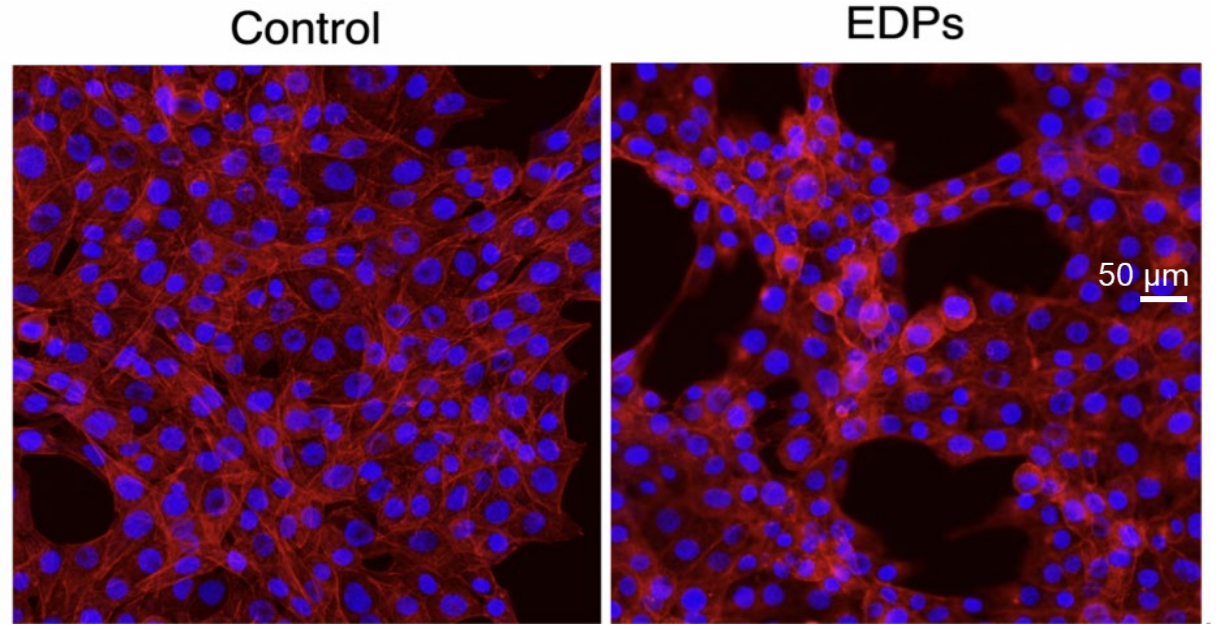

Figure 5. Cell Phalloidin Staining of HT-22 incubation with control and EDPs at 10 μM for 48h. Actin filaments are stained red. DAPI, a blue-florescent DNA stain is also applied to better visualize the position of each cell nucleus. Close up images are shown to better present details of the quantity and intensity of stress fibers.

3.4. Galactose and Chondroitin Sulfate Mitigates the Effects of EDPs

Addition of chondroitin sulfate and galactose with EDPs to HT-22 cell-lines shows reduced damage on cellular cytoskeleton (Fig. 6). Phalloidin staining images for both the chondroitin sulfate and galactose groups shows evident increase in actin density and stress fiber frequency. The overall shape is also comparatively less circular when compared to the pure EDPs groups. Though a difference still exists between the control and the two rescue groups, it is clear how the effects of EDPs on cellular cytoskeleton assembly can be reduced.

DOI: 10.54254/2753-8818/15/20240476

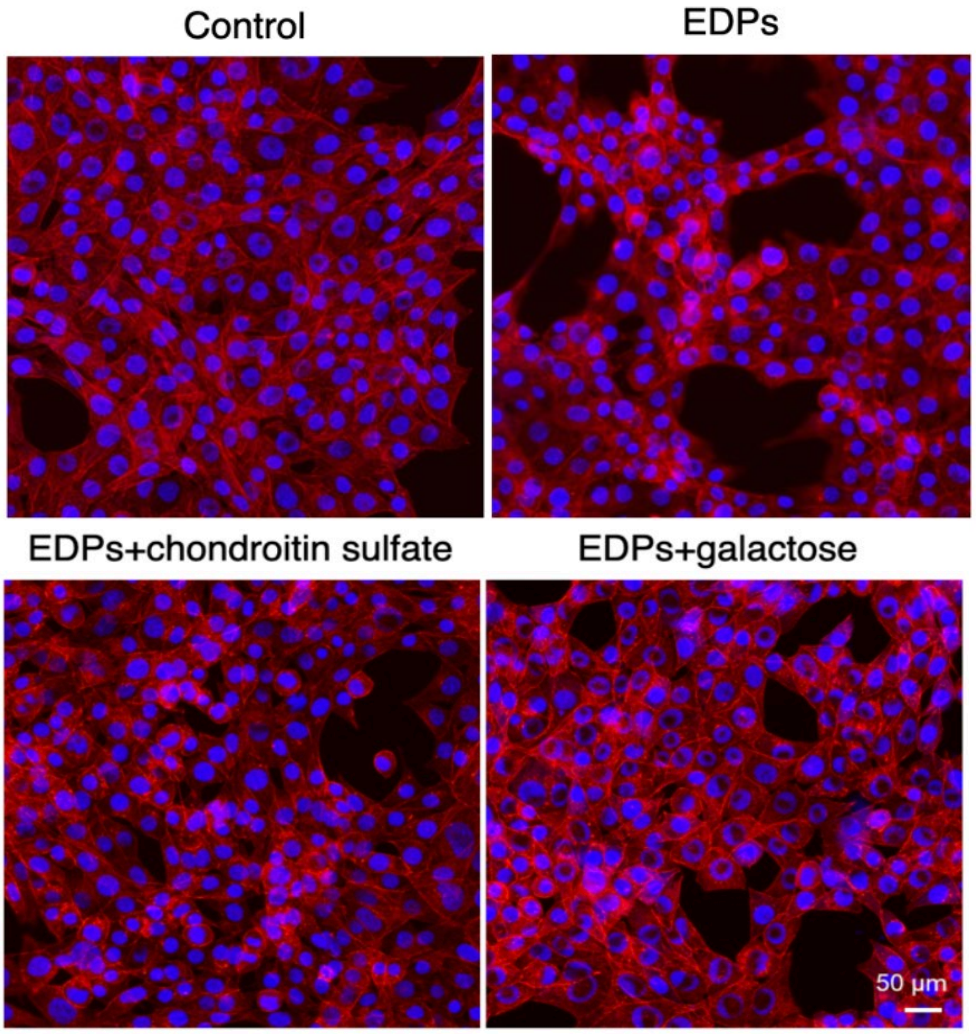

Figure 6. Rescue Experiment Phalloidin staining images. Actin filaments are stained red. DAPI, a blue-florescent DNA stain is also applied to better visualize the position of each nucleus. The above images for control and EDPs are the same as figure 5 and put as reference. The lower left image are cells incubated with 10 μM EDPs and 10 μM chondroitin sulfate. The lower right image are cells incubated with 10 μM EDPs and 20 μM galactose.

3.5. EDPs Decrease Phosphorylation Levels of Cofilin Proteins

Cofilin is an actin-binding protein responsible for the regulation and depolymerization of actin molecules. The over-activation of such proteins has been known to alter cell shape, and may causes to multiple diseases.

Western blotting data on the phosphorylation of cofilin protein suggests EDPs ability to up-regulate cofilin activity and stress cytoskeleton assembly. As seen in Fig.7 below, three western blotting trials for the overall cofilin level, phosphorated cofilin levels (p-cofilin), and β-actin levels for reference was taken for both the control and EDPs groups. First, the overall similar concentration of cofilin groups suggests that the amount of cofilin proteins present within each sample were similar. However, the p-cofilin levels are visibly darker for the three control samples compared to the EDPs groups, suggesting a higher overall percent of phosphorated cofilin proteins. Lastly, the similar intensity of β-actin control suggests that each trial was of a similar amount.

DOI: 10.54254/2753-8818/15/20240476

According to the data, EDPs decrease the overall phosphorylation levels of cofilin proteins, meaning more cofilin proteins in EDPs groups are activated than control and therefore over-modifying the preexisting f-actin and stress fibers. From a macro scale, this also explains the damage in cellular cytoskeleton present in the phalloidin staining data, suggesting the structural damage was indeed caused by abnormalities within cytoskeleton assembly due to increased levels of EDPs.

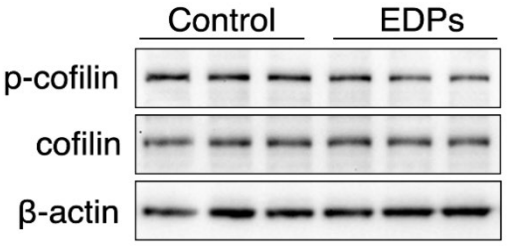

Figure 7. Western Blotting Data of cofilin phosphorylation. Western blotting of HT-22 cell lines tested for the concentration of phosphorated cofilin and cofilin concentrations for both control and EDPs groups. Three trials were taken for each, and each column being tests on the same trial. Concentration of β-actin molecules are also taken as control of the original concentration of cells in each.

4. Discussion

This research identified a comprehensive view on how EDPs influences neuronal functions and the subsequent progression of Alzheimer's Disease. From experimental data with HT-22 cell lines, EDPs have shown to influence both cell vitality and morphology, which are two vital factors influencing the overall functionality of neurons within the central nervous system. In addition, western blotting data also verifies the micro cause of overall distortion in cell shape to be an over-activation of cofilin proteins causing stress for f-actin molecules vital to cellular cytoskeleton shape. This also suggests the ability of extracellular EDPs to induce signaling within cells, verifying signaling pathways through membrane bound receptors.

Moreover, data collected overall matches with already existing understandings about the disease itself. Both the concentration of EDPs and duration of exposure increases through age, matching the current knowledge of AD mostly effecting the elderly. This also matches most preexisting studies used for the meta-analysis, confirming the overall accuracy of this study.

Two differences however do exist as outliers to the data collected. First, animal/cell experiments are fundamentally different from clinical studies. This is not only a scientific consensus but one visible between the studies used for the meta-analysis. Therefore, the lab portion of this research being mostly cell experiments does mean limitations when directly being applied to humans. Second, men and women are also fundamentally different with their responses to EDPs. As one article suggests, studies on women test subjects are more statistically significant than the same test on males. Women are also more likely to be affected by AD than their male counterparts. This suggests that in addition to the over-activated cofilin and cytoskeleton, there are other pathological effects of EDPs, which may be related to sex hormones. This is also another limitation within our study, suggesting more potential details would be addressed with how EDPs influences neuronal function.

Nevertheless, despite the acknowledged differences and limitations, this study also has its contributions to the study of AD progression. It was reported that elevated EDPs levels can be detected before AD cognitive impairment occurs. Since the correlation between rising levels of EDPs and the progression AD is verified, this study suggests a potential possibility of using EDPs as an early biomarker for future AD diagnosis. As stated before, AD has traditionally been diagnosed clinically, with no other clear testing method, especially in the early-onset when clinical symptoms have not yet appeared. The only possible biomarker present is the testing for amyloid-beta, which usually rises too late before patients are clinically diagnosed to have an effect. Therefore, with further research into EDPs

DOI: 10.54254/2753-8818/15/20240476

at progressing stages of AD, EDPs can potentially become an important biomarker to diagnose AD at earlier stages.

Lastly, this was also the first study that ever explored the role of extracellular EDPs in this alternation of cell morphology that may lead to a decrease in neuronal function via the deformation of cytoskeleton assembly. From western blotting data, the phosphorylation of cofilin proteins is directly linked to EDPs concentrations outside a cell. This suggests that functions within a neuron can be affected through extracellular receptors, indicating not only a deeper understanding of AD but potential ways to solve it. Since functions of EDPs needs to be induced through membrane bound receptors, by limiting the receptors available for EDPs to bind to, the effects of EDPs should subsequently also decrease. This is verified through the rescue experiments, indicating the addition of other molecules such as chondroitin sulfate and galactose in blocking EDPs activation. This can potentially become a major way to treat EDPs caused AD in the future, and further investigation can lead to breakthroughs for the treatment of AD.

5. Conclusion

This study explored the relationship between EDPs levels and the progression of AD, especially the specific pathways extracellular EDPs takes to induce a loss in neuronal productivity. The initial hypothesis states an overall negative impact of EDPs on cell vitality and morphology, both vital factors for neuronal health. Through both meta-analysis on preexisting studies and vitro experimentation on HT-22 cell lines, the original hypothesis has been confirmed. A significant correlation exists between an increase in EDPs and the progression of AD, with the specific cause of cytoskeleton malformation being the up regulation of cofilin proteins activity. Rescue experiments however also identified that this over-activation of cofilin molecules can be counter-acted. Specific chemical such as galactose and chondroitin sulfate can successfully block elastin receptor sites, preventing it from binding to EDPs and reducing its downstream effects. Most importantly, being the first to ever identify the above, this study brings new insight to future developments in AD identification and treatment.

For example, as stated in the discussion, a major field of further studies from these finding is the verification of EDPs as a biomarker for AD. Since EDPs circulates the entire body, easy blood sample test can be used to acquire data for EDPs either diagnosed clinically with AD or shows symptoms of it. It can also be compared to the amyloid-beta levels within the same patients to see variances between levels of both within patients that eventually develop AD symptoms. This can then give critical information on micro biological changes within patients and promptly treatment before irreversible damage is done to overall cognition.

Lastly, treatment for EDPs caused AD is also feasible. Since this lab identified a cause of AD to be extracellular influences, treatment such as blocking receptor sites can be achieved. Through further investigation into the specific mechanism of the mechanism from extracellular EDPs ligands to responses within cells, this type of AD can be completely prevented and cured.

References

[1] Heinz, Andrea. "Generation and Effects of Human Elastokines." Critical Reviews in Biochemistry and Molecular Biology, 12 June 2020. Accessed 6 Feb. 2023.

[2] Le Page, A. et al. "The role of elastin-derived peptides in human physiology and diseases. " Matrix biology: journal of the International Society for Matrix Biology, 84, 81–96. https://doi.org/10.1016/j.matbio.2019.07.004

[3] Kobayashi, K. et al. "Elastin derived peptides are involved in the processes of human temporomandibular disorder by inducing inflammatory responses in synovial cells" Mol Med Rep, 2017. 16(3): p. 3147-3154.

[4] Scheltens, P., De Strooper, B., Kivipelto, M., Holstege, H., Chételat, G., Teunissen, C. E., Cummings, J., & van der Flier, W. M. (2021). Alzheimer's disease. Lancet (London, England), 397(10284), 1577–1590. https://doi.org/10.1016/S0140-6736(20)32205-4

[5] Huang, Chin-Chia et al. “Studying Arterial Stiffness Using High-Frequency Ultrasound in Mice with Alzheimer Disease.” Ultrasound in medicine & biology vol. 43,9 (2017): 2054-2064. doi:10.1016/j.ultrasmedbio.2017.04.029

[6] Li, JingJing, et al. "Extracellular Elastin Molecule Modulates Alzheimer's Aβ Dynamics in Vitro and in Vivo by Affecting Microglial Activities."Chinese Chemical Society, vol. 3, no. 7, 2020, pp.1830-37, https://doi.org/10.31635/ccschem.020.202000330.

[7] Ma, Chao et al. “Significant Upregulation of Alzheimer's β-Amyloid Levels in a Living System Induced by Extracellular Elastin Polypeptides.” Angewandte Chemie (International ed. in English) vol. 58,51 (2019): 18703-18709. doi:10.1002/anie.201912399

[8] Moscarelli, Pasquale et al. “Structural characterization and biological properties of the amyloidogenic elastin-like peptide (VGGVG)3.” Matrix biology : journal of the International Society for Matrix Biology vol. 36 (2014): 15-27. doi:10.1016/j.matbio.2014.03.004

[9] Bizbiz, L et al. “Aging of the vascular wall: serum concentration of elastin peptides and elastase inhibitors in relation to cardiovascular risk factors. The EVA study.” Atherosclerosis vol.131,1 (1997): 73-8. doi:10.1016/s0021-9150(97)06076-0

[10] Gutierrez, Jose et al. “Brain arterial aging and its relationship to Alzheimer dementia.” Neurology vol. 86,16 (2016): 1507-15. doi:10.1212/WNL.0000000000002590

[11] Merlini, Mario et al. “Tau pathology-dependent remodelling of cerebral arteries precedes Alzheimer's disease-related microvascular cerebral amyloid angiopathy.” Acta neuropathologica vol. 131,5 (2016): 737-52. doi:10.1007/s00401-016-1560-2

[12] Nicoloff, G et al. “Detection of elastin derived peptides in cerebrospinal fluid of patients with first ever ischaemic stroke.” Neuropeptides vol. 42,3 (2008): 277-82. doi:10.1016/j.npep.2008.02.003

[13] Péterszegi, G et al. “Increased elastase and cathepsin G activity in activated lymphocytes from aged patients: role of denutrition and dementia.” Archives of gerontology and geriatrics vol. 25,3 (1997): 285-98. doi:10.1016/s0167-4943(97)00020-4

[14] Tzvetanov, P et al. “Increased levels of elastin-derived peptides in cerebrospinal fluid of patients with lacunar stroke.” Clinical neurology and neurosurgery vol. 110,3 (2008): 239-44. doi:10.1016/j.clineuro.2007.11.003

[15] Tzvetanov, Plamen et al. “Abnormal levels of serum anti-elastin antibodies in patients with symptomatic carotid stenosis.” Clinical neurology and neurosurgery vol. 116 (2014): 9-12. doi:10.1016/j.clineuro.2013.11.011