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CEREBROLYSIN® INFLUENCES IN *Sod*- AND *sws*-DEPENDENT NEURODEGENERATIVE MODELS OF *DROSOPHILA MELANOGASTER*

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Background. The incidence of human neurodegenerative disorders increases continuously as the human population ages. To date, these diseases remain incurable and require complex experimental approaches using tractable models to study the degeneration mechanisms and potential drug intervention regimens. In the current work, we assessed the impact of the neuroprotective drug Cerebrolysin on these neurodegenerative processes in *Drosophila Sod1* and *swiss cheese* (*sws*) mutants.

Materials and Methods. The experiments were conducted using a *D. melanogaster Sod1*- and *sws*-dependent neurodegeneration model. Cerebrolysin (3 μL/mL) was added for larvae feeding. In order to evaluate Cerebrolysin influence, several tests were performed: locomotor activity assay, lifespan, size of brain tissue degeneration zones and sensitivity to prooxidant exposion.

Results. Dietary supplementation with Cerebrolysin extended the lifespan of all flies under normal circumstances. The drug treatment also reduced the sensitivity of mutant flies to pro-oxidant effects. Moreover, treatment with Cerebrolysin partially diminished the size of degeneration zones in the brain tissue of sws^1 mutant flies, without any notable effects on locomotor ability.

Conclusions. The data obtained confirm the moderate neuroprotective and/or antioxidant action of Cerebrolysin against neurodegenerative processes under different genetic backgrounds.

Keywords: neurodegeneration, Cerebrolysin, lifespan, behavior, Cu-Zn superoxide dismutase *Sod1*, *swiss cheese* genes

INTRODUCTION

Neurodegenerative diseases encompass several disorders caused by genetic and exogenous factors. Although by this time, genes involved in the induction of most human neurodegenerative disorders have been described, the molecular and cellular mechanisms leading to the manifestation of these disorders remain largely unresolved. Besides genetic components, risk factors evolving from lifestyle and global aging of the population have made substantial contributions to the development of these pathologies (Deal *et. al*. 2019; Durães *et. al*, 2018). The study of pathological mechanisms leading to neurodegeneration and, most importantly, the development of possible therapeutic and preventive measures, require the use of neurodegenerative study models. In the last decades, researchers on *Drosophila melanogaster* largely contributed to the understanding of neurodegenerative processes (Deal *et. al*., 2019). 75% of disease genes have orthologs in the *Drosophila* genome, and both cellular and physiological disease-related processes are similar between flies and humans (Pandey *et. al*., 2011).

Neurodegenerative diseases are incurable and, unfortunately, most therapeutic approaches based on specific molecular targets have proved to be ineffective. Therefore, the development and use of nootropic drugs (i.e., non-specific action neuroprotectors) have been suggested as a more viable approach. Cerebrolysin is one of such drugs. This drug is made of swine brain extract and is composed of a mixture of poorly described peptides. Although manufacturers do not disclose the exact composition of these drugs, recent analysis of Cerebrolysin components indicated that they consist of approximately 14635 peptides serving different functions. Most of the peptides detected are probably activity modulators of signal proteins. These modulators include, in particular, kinases and proapoptotic caspases. The fact that Leu- and Met-encephalines, neuropeptides fragments, and neuronal growth factors are present in Cerebrolysin confirms the neurotrophic potential of these drugs (Tharwat *et. al*., 2023).

Clinical and research data on the role of Cerebrolysin on neuronal tissue health is contradictory, to some extent, and require more detailed studies, including those using biological disease models. In light of what is known about Cerebrolysin, it appears that the drug could help relieve symptoms associated with Alzheimer's disease and vascular dementia, according to the Alzheimer's Drug Discovery Foundation. *In vitro* studies using neuronal cell cultures show that the drug prevents damage to neurons and helps form new neuronal connections (Schauer *et. al*., 2006; Lucas *et al.,* 2014).

To study the impact of Cerebrolysin on neurodegenerative phenotype, we used two models of neurodegenerative disorders in *Drosophila melanogaster*: 1) a *Sod1*-dependent model as an example of antioxidant system dysfunction (Celotto *et. al.,* 2012; Vituschynska *et. al.,* 2015), and 2) *sws*-dependent model, which is an example of polyneuropathy with glia impairment and neuronal death (Kretchmar *et. al*., 1997; Ryabova *et. al*., 2018). The use of these model systems (*Sod1* and *sws* mutants) allowed us to comprehensively assess the effects of Cerebrolysin as a non-specific neuroprotective and neurotrophic drug.

MATERIALS AND METHODS

Fly Stocks and Conditions. All experiments were performed within *Drosophila melanogaster* flies with enhanced neurodegenerative changes in the brain. Mutants *swiss cheese* (*sws*[1]), a line kindly provided by Prof. Doris Kretzschmar (1997),

was isogenized to *Oregon-R* wild type line, which served as control. Gene knockdown of the Cu-Zn superoxide dismutase (*Sod1*) in glial cells – *repo>Sod1^{RNAi}* (Vituschynska *et. al.,* 2015) was obtained by crossing *repo-Gal4/TM3 (repo)* (kindly provided by Prof. Karl Fischbach) and *w*;*P{UAS-Sod.IR}4* (*UAS-Sod1^{RNAi}*) lines (Bloomington Drosophila Stock Center, USA (BDSC #24291)); we used heterozygous *Oregon/repo-Gal4* flies as a control. *Sod1* gene mutant (BDSC #24490 line) (Vituschynska *et. al.,* 2013) *Sod1^{X-39} e^{1}/TM3,Sb^{1}Ser^{1}* was crossed with *Oregon-R* for getting rid of balancers; in this case *e^{1}/Oregon-R* flies served as a control. Flies were kept in vials at 24 °C, on a standard medium (0.7% agar, sugar, yeast, raisins, and semolina 5% of each). Cerebrolysin® (Cerebrolysin) is a multi-modal neuropeptide drug with neurorecovery properties. Experimental Cerebrolysin concentration in the medium was estimated based on the recommended daily dose maximum of drug per 1 kg per 100 ml of medium (3 µL/mL) and fed during the larval stage only (Ashburner *et. al*., 1989, Matiytsiv, *et. al*., 2015). Adult flies were kept on the medium without the drug.

Food Intake quantitative assay. The amount of consumed food was calculated from the optical absorption of the consumed dye described before (Skorupa *et al*., 2008) with a little modification. Homogenate: 10 larvae were crushed in a homogenizer with the addition of 0.5 mL of 0.1 M Tris-HCl homogenate buffer. Homogenization was carried out in a cooled homogenizer at a temperature of 0–4 °C. After centrifugation (16,100 g, 15 min), 375 µL of the supernatant was mixed with 375 µL of homogenization medium and centrifuged again. Before measurement, 750 µL of homogenization medium was added to the collected supernatant to reach a volume of 1.5 mL. The calibration curve was constructed based on the optical absorbance of a solution of FD&C Blue N1 dye at a concentration of 0.8 to 16 µg/mL. The optical absorbance of supernatants and calibration samples was determined on a Denovix nanodrop at a wavelength of 629 nm.

Survival Assay. To generate survival curves, no less than 100 males of each genotype were used. Ten flies were housed per vial, live flies were counted and moved to fresh medium every 2–3 days. Results were processed using Graph Pad Prism 7 software (Graphpad Software Inc., La Jolla, CA, USA), and the reliability of data obtained was tested with a Log-rank test.

Histological sections and quantification of brain tissue degeneration Degenerative changes in the brain tissues were detected by analysis of histological sections prepared as previously described (Heisenberg *et. al*., 1979). Tissue degeneration was quantified by microphotography taken with Olympus IX73 microscope with DP-74 digital camera (Japan) with the use of green fluorescent light at 40× magnification. Brain tissues from 20 flies were analyzed from each group. Pictures were analyzed with the ImageJ software (https://imagej.nih.gov/ij). For each measurement, the total area of the lamina and medulla was selected separately. The area within those regions that correspond to vacuoles was determined as a percentage of the total area of the lamina or medulla respectively.

Climbing Activity Assay. Locomotor activity index (L index) was calculated by climbing test using phototaxis detection construction (Benzer, 1973) with some modifications (Greene *et. al.,* 2003; Matiytsiv, *et. al*., 2013). The construction comprised five consecutive vials. The number of males who climbed into the upper vial in 30 seconds was counted and the flies were transferred to the next vial with no resting time. After 30 seconds, males that remained in it were counted. Those flies that climbed into the upper were moved into the next vial and this continued up to the last vial in the installation.

The test was done using 13- to 15-day-old flies and about 80 flies were tested per genotype. The flies were divided into 3 groups of 20 males, and each group was tested three times. Tested flies were kept without anesthesia for at least 24 hours. L index was calculated using the following equation, where n_x – number of flies in a vial:

$$\text{L index} = \frac{(n_1) + (n_2 \cdot 2) + (n_3 \cdot 3) + (n_4 \cdot 4) + (n_5 \cdot 5)}{n \cdot 5}.$$

Resistance against prooxidant H_2O_2. Experiment was performed as described earlier (Mohylyak *et. al*.,2011). One hundred 3-day-old males were put into vials with agarose medium (10 flies per vial). Two hundred microliters of 5% H_2O_2 in 10% sucrose was applied to the filter; 10% sucrose solution was used for control. Every 24 hours, flies were moved to fresh vials. After 96 hours, the percentage of surviving flies in control and under prooxidant influence was calculated.

Statistical Analysis. Data were analyzed using GraphPad Prism 7 software. Statistical significance of the difference between groups was determined with ANOVA to determine statistical significance (p <0.05).

RESULTS

Quantitative consumption of Cerebrolysin. In order to make accurate comparisons among data sets, it is essential to determine whether individuals consume Cerobrolysin differently based on their genotype. It was determined that larvae of the *sws*[1] and *Sod1* mutants consume Cerebrolysin in the same amount as the control flies (**Fig. 1**).

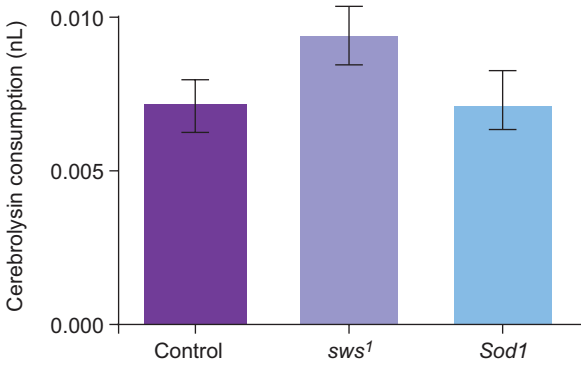

Fig. 1. Cerebrolysin intake of the larva. No significant difference: Control (n = 50), *sws*[1] (n = 50); *Sod1* (n = 50) (p = 0.2083, 0.9751)

Locomotor activity. Flies with mutations in *Sod1* and *sws* were shown to have a substantial decrease in locomotor activity (Matiytsiv *et. al*., 2013). The control flies had an L index of about 0.9, whereas in *Sod1* mutants it was lower by 50% and in *sws*[1] mutants by 75% (**Fig. 2**). Unfortunately, dietary supplementation with Cerebrolysin did not restore the locomotor activity.

The effect of Cerebrolysin feeding on fly survival. We drew survival curves of control flies and flies under experiments grown in standard medium and after Cerebrolysin feeding (**Fig. 3**). The chosen model systems turned out to be suitable for the study of this phenotype because *sws*[1] and flies with *Sod1* gene knockdown in glia cells had lower lifespans than controls (**Fig. 3**). We determined that Cerebrolysin

feeding prolonged the lifespan of all flies: controls and those displaying degeneration of both types (*Sod1*- and *sws*-depended) (**Fig. 3**). A more prominent effect was obtained on controls and flies with the *Sod1*-related model, which cannot be explained solely by Cerebrolysin effects. This incremental effect is likely to be contributed by the genotypic background of these lines.

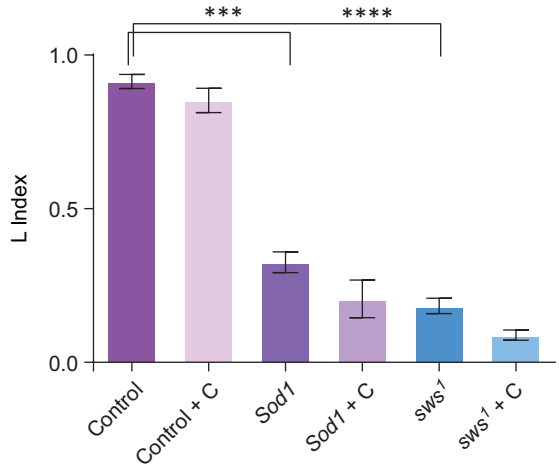

Fig. 2. Locomotor activity index. Cerebrolysin feeding (+C) did not change climbing activity of control flies n = 81, control + C n = 82 (p = 0.1896), *Sod1* n = 83, *Sod1* + C n = 82 (p = 0.1151) and sws^1 n = 79, sws^1 + C n = 78 (p = 0.6343). *** indicates significant difference at p ≤0.001; **** p ≤0.0001

Brain tissue neurodegeneration. Histopathological examination of the brain structure can inform of substantial degenerative changes caused by glial and neuronal cell death in the tissue. As described in **Fig. 4*B*** and **4*C***, regions of degeneration are represented by dark "vacuoles" in the brain tissue. Flies with *Sod1* gene knockdown in glia (*repo*>$Sod1^{RNAi}$) had small vacuoles in the lamina region (La) bordering the retina (Re), where about 3% of the tissue was degenerated (**Fig. 4*B*, 4*D***). Flies of sws^1 mutant displayed widespread brain tissue degeneration and larger degeneration zones (**Fig. 4*C***). Mutation induced degeneration of 17% in the lamina and 11% in the medulla (**Fig. 4*C*, 4*E***).

Cerebrolysin treatment did not induce any significant changes in the control flies (**Fig. 4*A***). Since flies of both neurodegeneration models showed unchanged brain phenotypes after Cerebrolysin feeding, we quantified the size of degeneration zones to estimate the possible neuroprotective effect of the drug. Dietary supplementation with Cerebrolysin was not effective to rescue changes induced by *Sod1* knockdown or *sws* mutation in the lamina (**Fig. 4*D*, 4*E***). However, Cerebrolysin treatment decreased the neurodegeneration in the medulla of *sws* mutant flies by 3% (p=0.009) (**Fig. 4*E***).

Survival under pro-oxidant exposure conditions. Increased sensitivity to pro-oxidants such as hydrogen peroxide in a short-term test is one of the specific features of flies with *Sod* gene dysfunction (Vituschynska *et. al.,* 2013). Over a four-day period of H_2O_2 exposure, *Sod1* mutants survived in significantly lower numbers than wild-type flies (72% and 90% respectively); the percentage of surviving *Sod1* mutants without pro-oxidant exposure was 90% (**Fig. 5*A*, 5*B***). After Cerebrolysin drug feeding, no changes were observed in survival indicators in all flies at standard conditions within 96 hours (**Fig. 5*A*, 5*B***). However, pro-oxidant sensitivity of *Sod1* mutants fed with Cerebrolysin dropped significantly and survival after H_2O_2 exposure approached the control level (up to 86%) (**Fig. 5*B***).

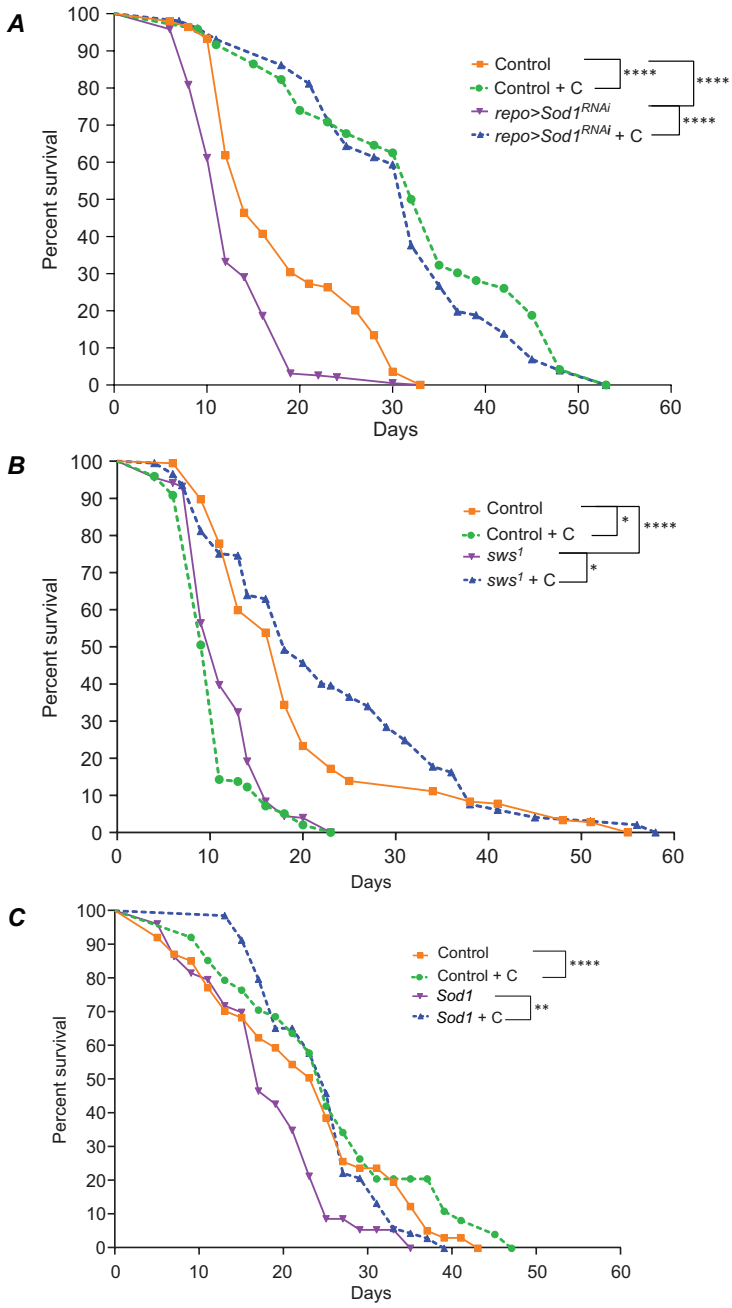

Fig. 3. Survival curves of flies grown on standard medium (regular lines on the graph) and after Cerebrolysin feeding (+C, dashed lines); Cerebrolysin significantly increased the lifespan of all flies: (***A***) *repo>Sod1*^{RNAi} flies with *Sod1* gene knockdown in glia cells compared with control *Oregon/repo-Gal4* (control n = 194, *repo>Sod1*^{RNAi} n = 193, control + C n = 96, *repo>Sod1*^{RNAi} + C n = 101, p <0.0001****); (***B***) *sws*^{1} mutants compared with control *Oregon-R* (control n = 185, *sws*^{1} n = 196, p <0.0001****, control + C n = 202, *sws*^{1} + C, n = 204 p = 0.0151*, p = 0.0113*); (***C***) *Sod1* mutants compared with control *e*^{1}*/Oregon-R* (control n = 101, *Sod1* + C n = 69, control + C n = 102, *Sod1* n = 103 p = <0.0001****, p = 0.0013**)

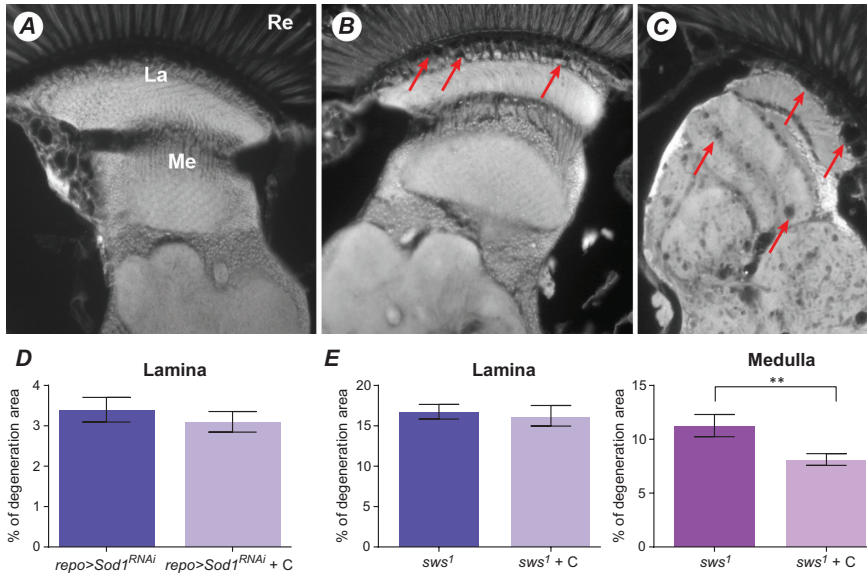

Fig. 4. Brain tissue characteristics on histological paraffin sections of (***A***) control flies, (***B***) flies with *Sod1* gene knockdown in glial cells *(repo>Sod1^{RNAi})*, and in (***C***) *sws^{1}* mutants. The quantitative analysis of degeneration zones is shown for (***D***) *repo>Sod1^{RNAi}* flies in the lamina region at standard conditions and after Cerebrolysin feeding (+C) (p = 0.4614), and for (***E***) of *sws^{1}* mutants before and after Cerebrolysin treatment (+C) in the lamina and the medulla regions (p = 0.8406 and p = 0.0024** respectively). n (of all flies types) = 20. Re – retina, La – lamina, Me – medulla

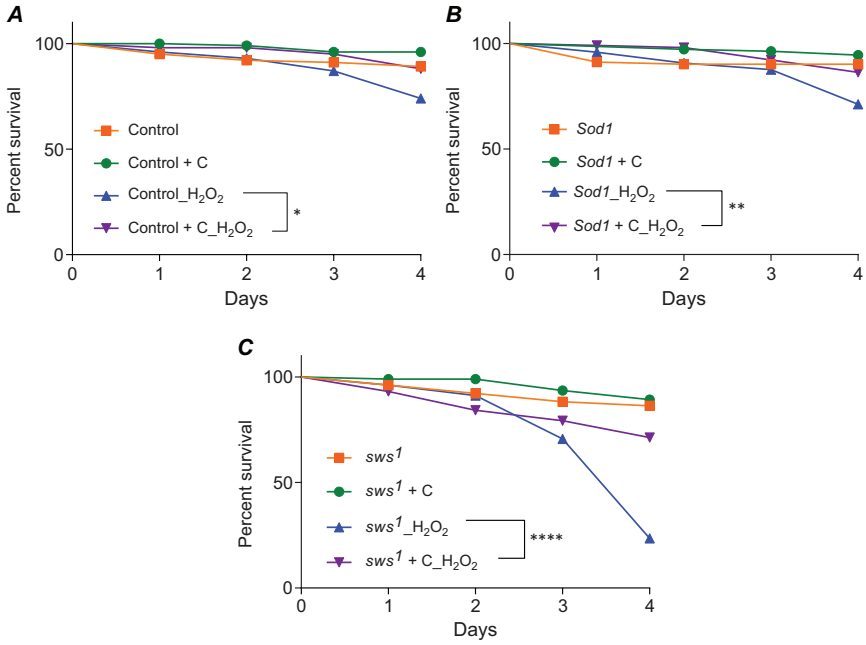

Fig. 5. Four-day survival of control and mutant flies at standard conditions and after H_2O_2 pro-oxidant exposure (***A***) Control flies: n = 101,+C n = 100,_H_2O_2 n = 99, +C_H_2O_2 n = 100 (P = 0.0106)*; (***B***) *Sod1* mutants: n = 100,+C n = 101, _H_2O_2 n = 101, +C_H_2O_2 n = 101 (P=0.0098**); (***C***) *sws^{1}* mutants: n = 102, +C n = 93, _H_2O_2 n = 100, +C_H_2O_2 n = 101 (P <0.0001****)

After 96 hours of H_2O_2 exposure, *sws*^{1} flies survived in significantly lower numbers than wild-type flies (74% and 29% respectively) while the survival index for *sws*^{1} mutants without pro-oxidant exposure was 86.6% (**Fig. 5*A*, 5*C***). After Cerebrolysin drug feeding, no changes were observed in survival indicators in all flies at standard conditions within 96 hours (**Fig. 5*C***). However, pro-oxidant sensitivity of *sws*^{1} flies fed with Cerebrolysin dropped up to 69% (**Fig. 5*C***).

DISCUSSION

Since Cerebrolysin may produce non-specific, general neuroprotective or neurotrophic effects, neurodegenerative model systems can help dissect the nature of these neuronal effects. To study the impact of Cerebrolysin on the brain tissue, we used two model systems of neurodegenerative disorders in *Drosophila melanogaster*. The systems consisted of models with *Sod1*-dependent mechanisms and those with *sws*-dependent mechanisms. *Sod1* and *sws* mutants, as well as flies with *Sod1* gene knockdown in glial cells (*repo>Sod1*^{RNAi}), showed degenerative changes in the brain (Vituschynska *et. al.,* 2015; Kretchmar *et. al*., 1997; Ryabova *et. al*., 2018). However, the mechanisms inducing neurodegenerative phenotypes in these two models differ from one another. The *Sod1* model is based on Cu-Zn superoxide dismutase enzyme impairment and malfunction of the cell antioxidant defense system, while *sws* mutation-induced changes appear to be due to glial and neuronal cell broken integrity.

Lifespan abbreviation is an important consequence of neurodegeneration in flies and humans. Thus, it becomes important to assess the effect of Cerebrolysin on the lifespan of the flies to assess the neuroprotective effects of the drug. A positive effect of Cerebrolysin on lifespan does not necessarily confirm its neuroprotective effect, as viability is a complex phenotype and does not depend exclusively on the conditions of brain cells alone. Therefore, we investigated the impact of Cerebrolysin on aged brain tissue. We hypothesized that the non-specific neuroprotective effect of the peptide drug can be feasible through its effects on glial cells. Therefore, we chose to study a form of degeneration with changes occurring in glial cells (i.e., *Sod1* gene knockdown in glial cells (Vituschynska *et. al.,* 2015)) and *sws*-dependent neurodegeneration at which the pathological glial structure was associated with neuronal death (Kretchmar *et. al*., 1997; Ryabova *et. al*., 2018). The region of the highest glial cell concentration on histological sections is localized on the retina – lamina boundary and to a lesser extent – on the lamina – medulla boundary (Mohylyak *et. al*., 2017). The fact that upon treatment with the Cerebrolysin drug the size of the vacuoles in the lamina did not change suggests that Cerebrolysin does not have a strong effect on the condition of glial cells. In contrast, the medulla is characterized by the greatest number of neuronal offshoots, and fewer glial cells and neuronal bodies. The reduction in the degeneration zones in the medulla of *sws*^{1} mutants after Cerebrolysin feeding is indicative of either a complex protective effect on all mentioned anatomic components of the medulla or one of them.

Lifespan and brain tissue changes are both considered basic phenotypical characteristics of neurodegeneration (Durães *et. al*, 2018; Deal *et. al*. 2019; Pandey *et. al*., 2011). When estimating the effect of neuroprotective drugs, it is necessary to determine their potential influence on behavioral changes. Behavioral changes are important symptoms that can adversely affect the quality of life in humans suffering from neurodegenerative diseases. Locomotor behavior is a fundamental behavior reaction that deter-

mines many other more complex forms of behavior. Therefore, locomotor activity is frequently estimated when studying neurodegeneration. The observed lack of any effect on locomotor activities in any of the flies under study may indicate that Cerebrolysin does not have any significant impact on the condition of neurons and their offshoots directly involved in behavior reactions.

In all studied flies we were able to show that these mutant flies became resistant to prooxidant action after Cerebrolysin feeding. This effect of Cerebrolysin can be explained either by the drug's ability to strengthen the antioxidant defense, as described by several authors (Tharwat *et. al*., 2023), or by an increased resistance to stress factors as a consequence of the general neuroprotective effect.

In this study, the positive phenotypic impacts of Cerebrolysin were observed for viability, prooxidant influence resistance, and degeneration of brain tissue. Future experiments will be needed to determine the mechanism through which Cerebrolysin may act.

CONCLUSION

Finding a cure for neurodegenerative disorders remains a significant challenge for current biomedical research. One of the suggested strategies, known as drug repositioning or drug reprofiling, offers to test existing drugs for neurodegenerative diseases (Durães *et. al*, 2018). Peptide neuroprotectors, to which Cerebrolysin belongs, remain poorly studied regarding their possible effects on neurodegenerative processes. Using the method of larvae feeding, we studied the effect of Cerebrolysin on important phenotypical manifestations of neurodegenerative changes: locomotor activity assay, lifespan, size of brain tissue degeneration zones and sensitivity to prooxidant explosion in *D. melanogaster* flies with different neuronal degeneration model systems – *Sod1*- and *sws*-dependent. The data we have obtained provide evidence of neuroprotective and/or antioxidant effects of Cerebrolysin, although a specific mechanism of action has not been determined. It is possible that the observed extension of the lifespan, the increased resistance to prooxidant effects, and the slight reduction in the degeneration zones in brain tissue occurred as a result of the combined effects of the multiple compositions of Cerebrolysin on different cell processes. Some of these cellular effects may include an increase in antioxidant protection and the number of neuronal connections and/or their damage prevention under neurotrophic factors as well as a strengthening of trophic and protective functions of glial cells is possible. Further study of Cerebrolysin's effects is needed to determine the most relevant functional mechanisms affected by the individual components of Cerebrolysin.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

All authors confirm that they have no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization, [N.M.]; methodology, [N.M.]; validation, [N.M.]; formal analysis, [NM].; investigation, [A.R.; Kh.D.; Z.N.]; resources, [N.M.]; data curation, [N.M.; A.R.]; writing – original draft preparation, [N.M.; A.R.]; writing – review and editing, [N.M.]; visualization, [N.M.; A.R.]; supervision, [N.M.]; project administration, [N.M.]; funding acquisition, [N.M.].

All authors have read and agreed to the published version of the manuscript.

REFERENCES

Alzheimer's Drug Discovery Foundation, Cerebrolysin (2016). New York. Retrieved from https://www.alzdiscovery.org/uploads/cognitive_vitality_media/addf-cerebrolysin-full-report.pdf

Ashburner, M. (1989). *Drosophila. A laboratory manual.* New York: Cold Spring Harbor Laboratory Press.
Crossref

Benzer, S. (1973). Genetic dissection of behavior. *Scientific American*, 229(6), 24–37. doi:10.1038/scientificamerican1273-24
Crossref ● PubMed ● Google Scholar

Celotto, A. M., Liu, Z., VanDemark, A. P., & Palladino, M. J. (2012). A novel *Drosophila* SOD2 mutant demonstrates a role for mitochondrial ROS in neurodevelopment and disease. *Brain and Behavior*, 2(4), 424–434. doi:10.1002/brb3.73
Crossref ● PubMed ● PMC ● Google Scholar

Deal, S. L., & Yamamoto, S. (2019). Unraveling novel mechanisms of neurodegeneration through a large-scale forward genetic screen in *Drosophila*. *Frontiers in Genetics*, 9, 700. doi:10.3389/fgene.2018.00700
Crossref ● PubMed ● PMC ● Google Scholar

Durães, F., Pinto, M., & Sousa, E. (2018). Old drugs as new treatments for neurodegenerative diseases. *Pharmaceuticals* (*Basel, Switzerland*), 11(2), 44. doi:10.3390/ph11020044
Crossref ● PubMed ● PMC ● Google Scholar

Greene, J. C., Whitworth, A. J., Kuo, I., Andrews, L. A., Feany, M. B., & Pallanck, L. J. (2003). Mitochondrial pathology and apoptotic muscle degeneration in *Drosophila parkin* mutants. *Proceedings of the National Academy of Sciences*, 100(7), 4078–4083. doi:10.1073/pnas.073755610
Crossref ● PubMed ● PMC ● Google Scholar

Heisenberg, M., & Böhl, K. (1979). Isolation of anatomical brain mutants of *Drosophila* by histological means. *Zeitschrift Für Naturforschung C*, 34(1–2), 143–147. doi:10.1515/znc-1979-1-228
Crossref ● Google Scholar

Kretzschmar, D., Hasan, G., Sharma, S., Heisenberg, M., & Benzer, S. (1997). The *swiss cheese* mutant causes glial hyperwrapping and brain degeneration in *Drosophila*. *The Journal of Neuroscience*, 17(19), 7425–7432. doi:10.1523/jneurosci.17-19-07425.1997
Crossref ● PubMed ● PMC ● Google Scholar

Lucas, B., Pinkernelle, J., Fansa, H., & Keilhoff, G. (2014). Effects of cerebrolysin on rat Schwann cells *in vitro. Acta Histochemica*, 116(5), 820–830. doi:10.1016/j.acthis.2014.01.013
Crossref ● PubMed ● Google Scholar

Matiytsiv, N. P., Dronska, K. A., Artymovych, N. M., Makarenko, A. M., & Chernik, Ya. I. (2015). Therapeutic effect of cerebral on neurodegeneration caused by altered functioning of *swiss cheese* gene in *Drosophila melanogaster. Studia Biologica,* 9(2), 99–106 doi:10.30970/sbi.0902.406 (In Ukrainian)
Crossref ● Google Scholar

Matiytsiv, N. P, Mohylyak, I. I., Truhs, O. I., Chernyk, Y. I. (2013). Rukhova aktyvnist neirodeheneratyvnykh mutantiv *Drosophila melanogaster* [Motor activity of *Drosophila melanogaster* neurodegenerative mutants]. *Odesa National University Herald. Biology*, 2(31), 70–76. doi:10.18524/2077-1746.2013.2(31).44771 (In Ukrainian)
Crossref ● Google Scholar

Mohylyak, I. I., Matiytsiv, N. P., Hrunyk, N. I., & Chernyk, Ya. I. (2011). Sensitivity of neurodegenerative mutants of *Drosophila melanogaster* from *Swiss cheese* group to the oxidative stress conditions. *Biopolymers and Cell*, 27(6), 453–458. doi:10.7124/bc.000117
Crossref ● Google Scholar

Mohylyak, I. I., & Chernyk, Ya. I. (2017). Functioning of glia and neurodegeneration in *Drosophila melanogaster*. *Cytology and Genetics*, 51(3), 202–213. doi:10.3103/s0095452717030094
Crossref ● Google Scholar

Pandey, U. B., & Nichols, C. D. (2011). Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery. *Pharmacological Reviews*, 63(2), 411–436. doi:10.1124/pr.110.003293
Crossref ● PubMed ● PMC ● Google Scholar

Ryabova, E., Matiytsiv, N., Trush, O., Mohylyak, I., Kislik, G., Melentev, P., & Sarantseva, S. (2018). *Swiss cheese*, *Drosophila* ortholog of hereditary spastic paraplegia gene *NTE*, maintains neuromuscular junction development and microtubule network. In F. K. Perveen (Ed.), *Drosophila melanogaster – model for recent advances in genetics and therapeutics* (pp. 209–225). Rijeka, Croatia: IntechOpen. doi:10.5772/intechopen.73077
Crossref ● Google Scholar

Schauer, E., Wronski, R., Patockova, J., Moessler, H., Doppler, E., Hutter-Paier, B., & Windisch, M. (2006). Neuroprotection of Cerebrolysin in tissue culture models of brain ischemia: post lesion application indicates a wide therapeutic window. *Journal of Neural Transmission*, 113(7), 855–868. doi:10.1007/s00702-005-0384-3
Crossref ● PubMed ● Google Scholar

Skorupa, D. A., Dervisefendic, A., Zwiener, J., & Pletcher, S. D. (2008). Dietary composition specifies consumption, obesity, and lifespan in *Drosophila melanogaster*. *Aging Cell*, 7(4), 478–490. doi:10.1111/j.1474-9726.2008.00400.x
Crossref ● PubMed ● PMC ● Google Scholar

Tharwat, E. K., Abdelaty, A. O., Abdelrahman, A. I., Elsaeed, H., Elgohary, A., El-Feky, A. S., Ebrahim, Y. M., Sakraan, A., Ismail, H. A., Khadrawy, Y. A., Aboul Ezz, H. S., Noor, N. A., Fahmy, H. M., Mohammed, H. S., Mohammed, F. F., Radwan, N. M., & Ahmed, N. A. (2023). Evaluation of the therapeutic potential of cerebrolysin and/or lithium in the male Wistar rat model of Parkinson's disease induced by reserpine. *Metabolic Brain Disease*. doi:10.1007/s11011-023-01189-4
Crossref ● PubMed ● Google Scholar

Vitushynska, M., Matiytsiv, N., & Chernyk, Ya. (2013). Chutlyvist do umov oksydatyvnoho stresu, tryvalist zhyttia ta neirodeheneratyvni zminy v strukturi mozku u mutantiv *Drosophila melanogaster* za henamy superoksyddysmutazy [Sensitivity to the oxidative stress conditions lifespan and neurodegenerative changes in the brain structure of *Drosophila melanogaster* superoxiddismutase mutants]. *Visnyk of the Lviv University. Series Biology*, 62, 108–116. (In Ukrainian)
Google Scholar

Vitushynska, M. V., Matiytsiv, N. P., & Chernyk, Y. I. (2015). Influence of tissue-specific superoxide dismutase gene expression in brain cells on *Drosophila melanogaster* sensitivity to oxidative stress and viability. *Cytology and Genetics*, 49(2), 95–101. doi:10.3103/s0095452715020127
Crossref ● PubMed ● Google Scholar

ВПЛИВ ЦЕРЕБРОЛІЗИНУ® за *Sod- і sws*-ЗАЛЕЖНОЇ НЕЙРОДЕГЕНЕРАЦІЇ У *DROSOPHILA MELANOGASTER*

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Вступ. Зі старінням населення кількість людей із нейродегенеративними розладами постійно зростає. На сьогоднішній день ці захворювання залишаються невиліковними, це потребує експериментальних підходів із використанням відповідних модельних систем для вивчення механізмів дегенерації та потенційних способів медикаментозної допомоги. У цій роботі ми дослідили вплив нейропротекторного препарату Церобролізин на нейродегенеративні процеси в мутантів *Drosophila* за генами *Sod1* і *swiss cheese* (sws).

Матеріали та методи. Експерименти проводили на *D. melanogaster Sod1* та *sws*-залежній моделі нейродегенерації. Личинкам додавали до раціону Церебролізин (3 мкл/мл). Для аналізу впливу Церебролізину було проведено тести на визначення рухової активності, тривалості життя, розміру зон дегенерації тканини головного мозку та чутливості до дії прооксидантів.

Результати. Споживання Церебролізину подовжило тривалість життя всіх мух за нормальних умов та знизило чутливість мух із нейродегенерацією до впливу прооксиданта. Крім того, застосування Церебролізину частково зменшило розмір зон дегенерації в мозку мух *sws*^{1} без будь-якого помітного впливу на рухову активність.

Висновки. Отримані дані вказують на помірну нейропротекторну та/або антиоксидантну дію Церебролізину за нейродегенеративних процесів різного походження.

Ключові слова: нейродегенерація, Церебролізин, тривалість життя, поведінка, Cu-Zn супероксиддисмутаза *Sod1*, *swiss cheese* гени

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