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The influence of Mitochondrin-2 and Cerebrolysin on the survival of Drosophila melanogaster individuals with the functional knockout of Sod1gene in glia

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INTRODUCTION

- Age dependent degeneration in the adult nervous system is often characterized by a spectrum of abnormalities.
- *Drosophila* model is a very attractive system to study human neurodegenerative disorders.
- The functional knockout of Sod1 Drosofila melanogaster gene in glia (UAS-Sod1-RNAi/Repo-Gal4) causes degenerative changes in the brain and decrease in viability.
- Using invertebrate models as *Drosophila* in finding therapies against human diseases offers a wide range of advantages.

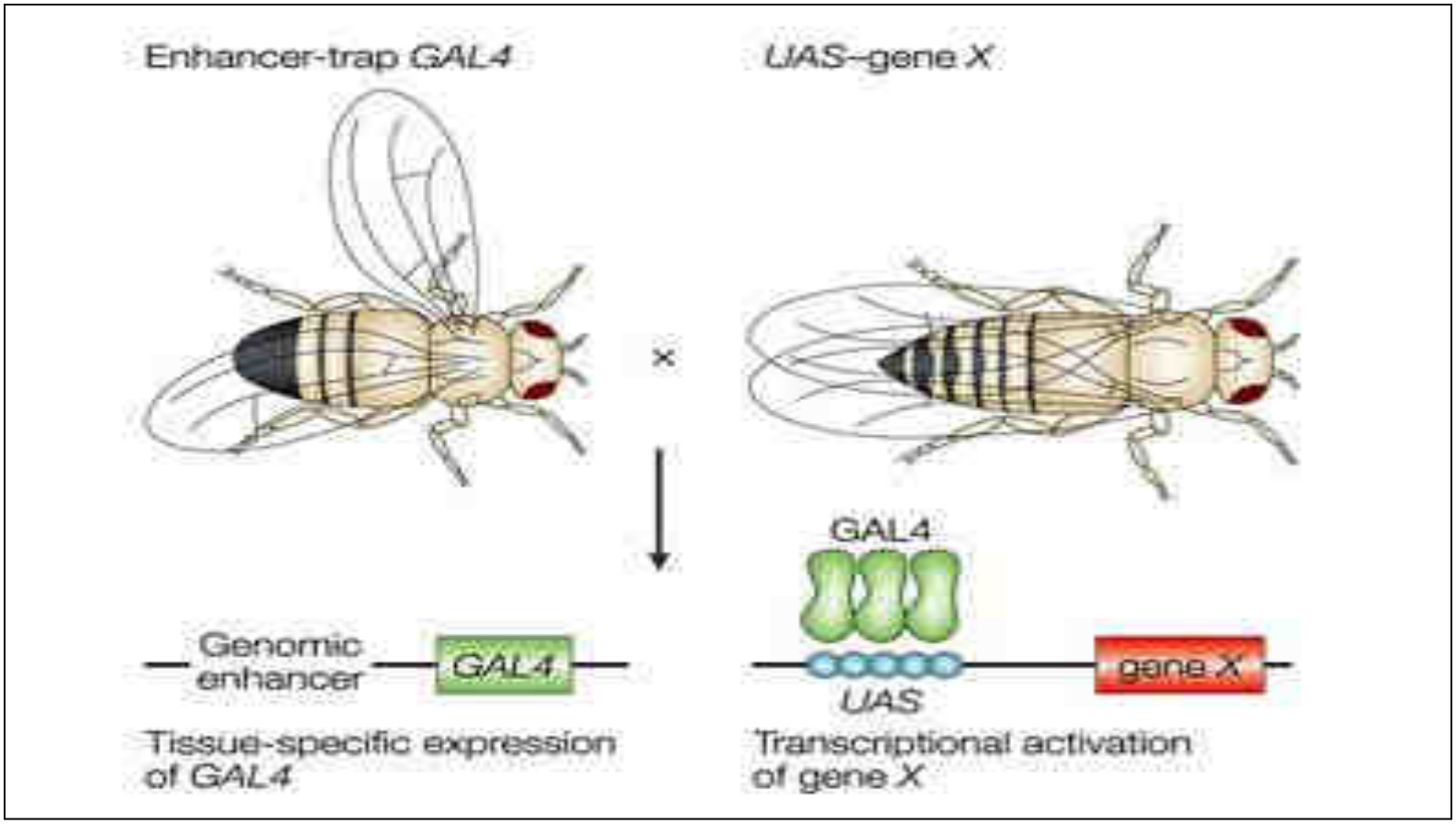
AIM OF STUDY

We studied the influence of the experimental agent of Mitochondrin-2 (M-2) and Cerobrolysin (“Ever”, Austria) (C) on the viability of Drosophila UAS-Sod1-RNAi/Repo-Gal4 individuals under standard conditions and during oxidative stress.

METHODS

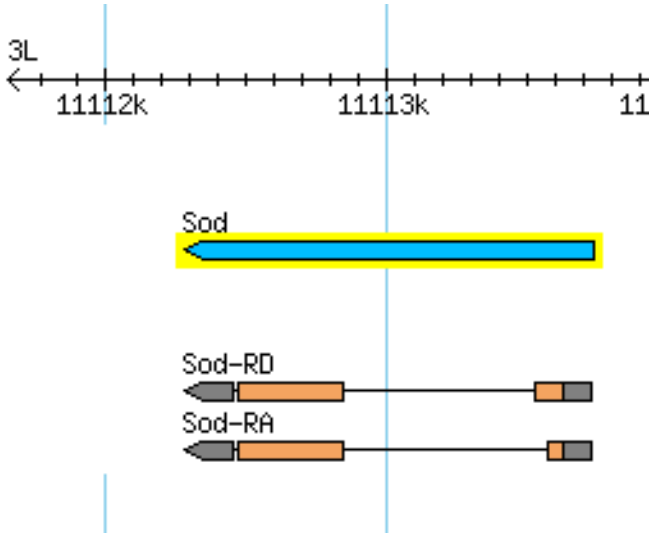
- Using the Drosophila UAS-Gal4 system of gene expression, we created a specific functional knockout of gene Sod1 in astroglial cells.
- The detoxication of free radicals is carried out by the antioxidant system of protection, which starting enzyme is superoxide dismutase (SOD).
- Therapeutic influence was assessed in different age of imago stage of flies by paraffin brain sections in ultraviolet (UV) light and lifespan analyzing.
- Mitohondrin-2 (M2) was created from mitochondrial fractions of cerebral cortex tissue of the post-hypoxia newborn piglets. Cerebrolysin (C) (Ever, Austria) was obtained from brain of adults pigs. We used M2 and C for treatment of flies by method of larval feeding.
- 5% H_2O_2 solution was used as a prooxidant.

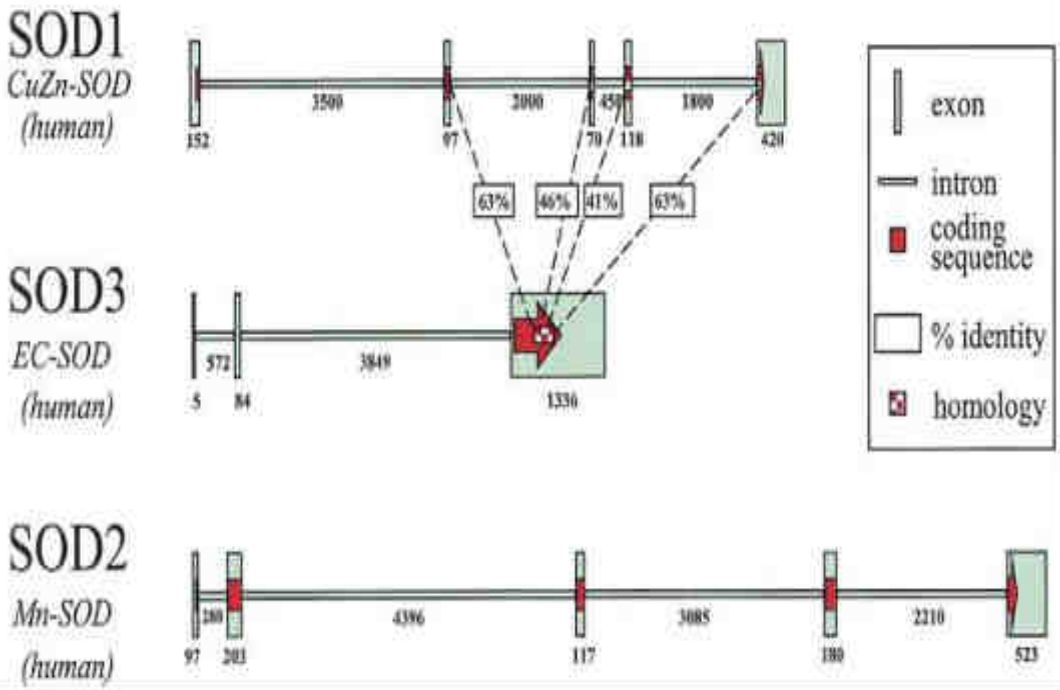
UAS-Gal4 binary system



Brand A., Perrimon N.// Development. 1993. V.118(2).P. 401–415.

Sod1


3L
11112k
11113k
11
Sod
Sod-RD
Sod-RA


SOD1
CuZn-SOD
(human)
SOD3
EC-SOD
(human)
SOD2
Mn-SOD
(human)
exon
intron
coding sequence
% identity
homology

TWO MAIN PHASES OF CELLS' REACTION UNDER TISSUES ALTERATION (IADECOLA C., 2015)

PHASE OF TISSUE DESTROY:

I. 1. Genes of early reaction,
Stress-proteins (HSPs).

2. Glutamate "excite toxicity", growth of Ca2+ content inside of the cell, lactate acidosis.

3. NOS, COX-2 (inductors of the oxidant stress).

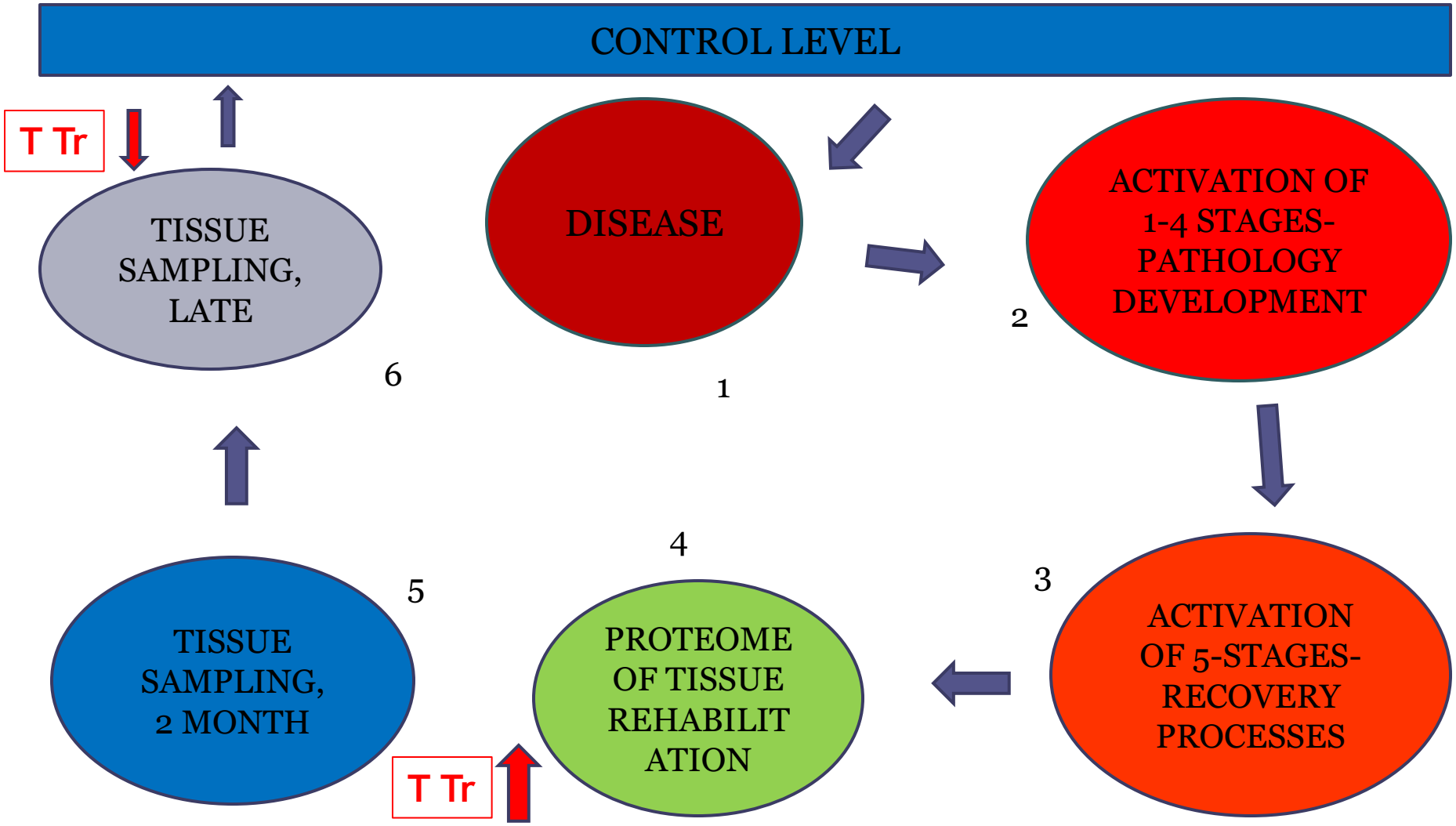
4. Cytokines and molecules of intercellular adhesion, genetic programs of "inflammation" and "apoptosis".

PHASE OF TISSUE RECOVERY:

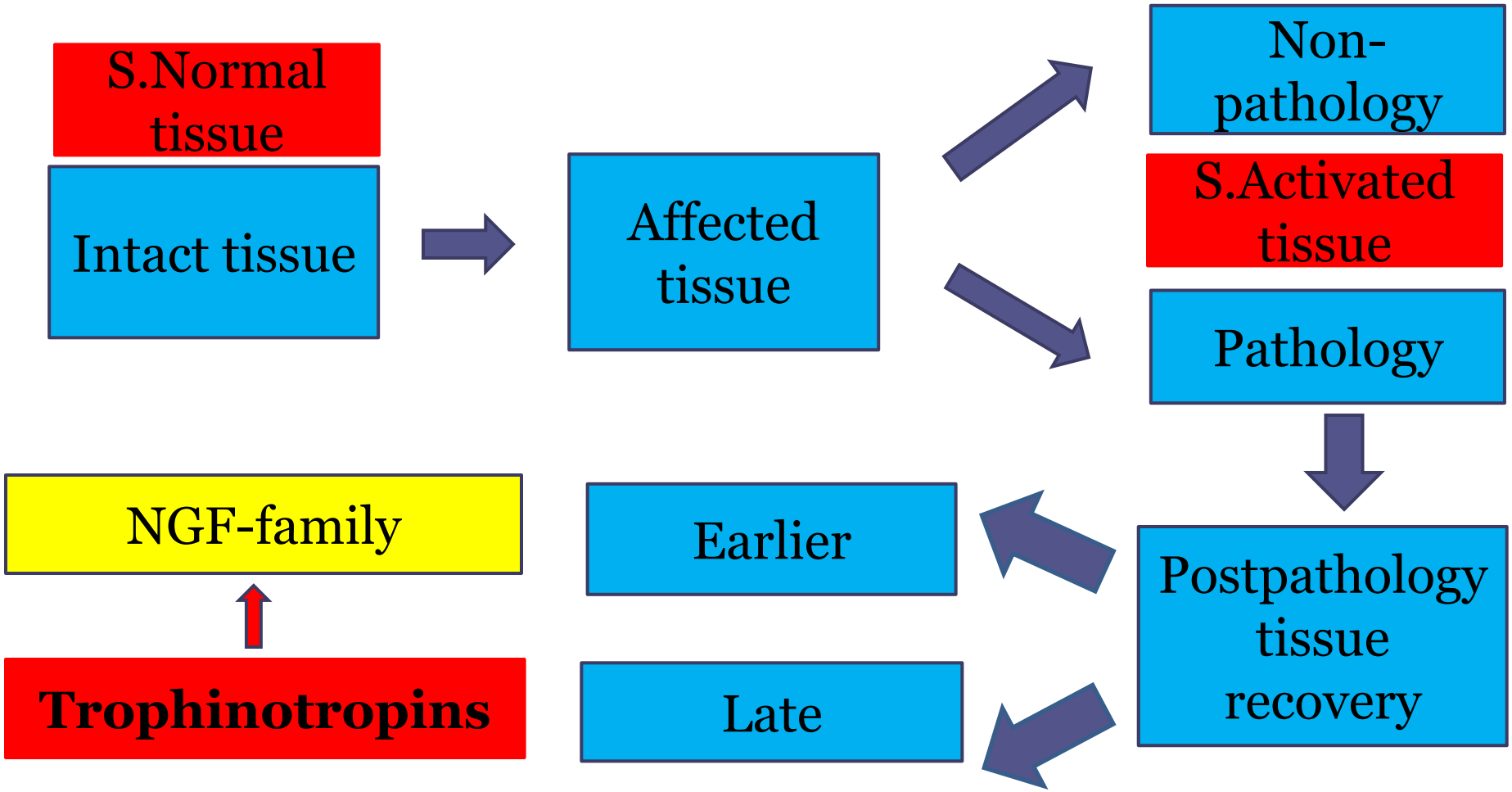
II. 5. Genetic programs of reparation (neurotrophins, other factors of growth, Cerebral, Adement, Postrong, M2),

6. Growth phosphoproteins.

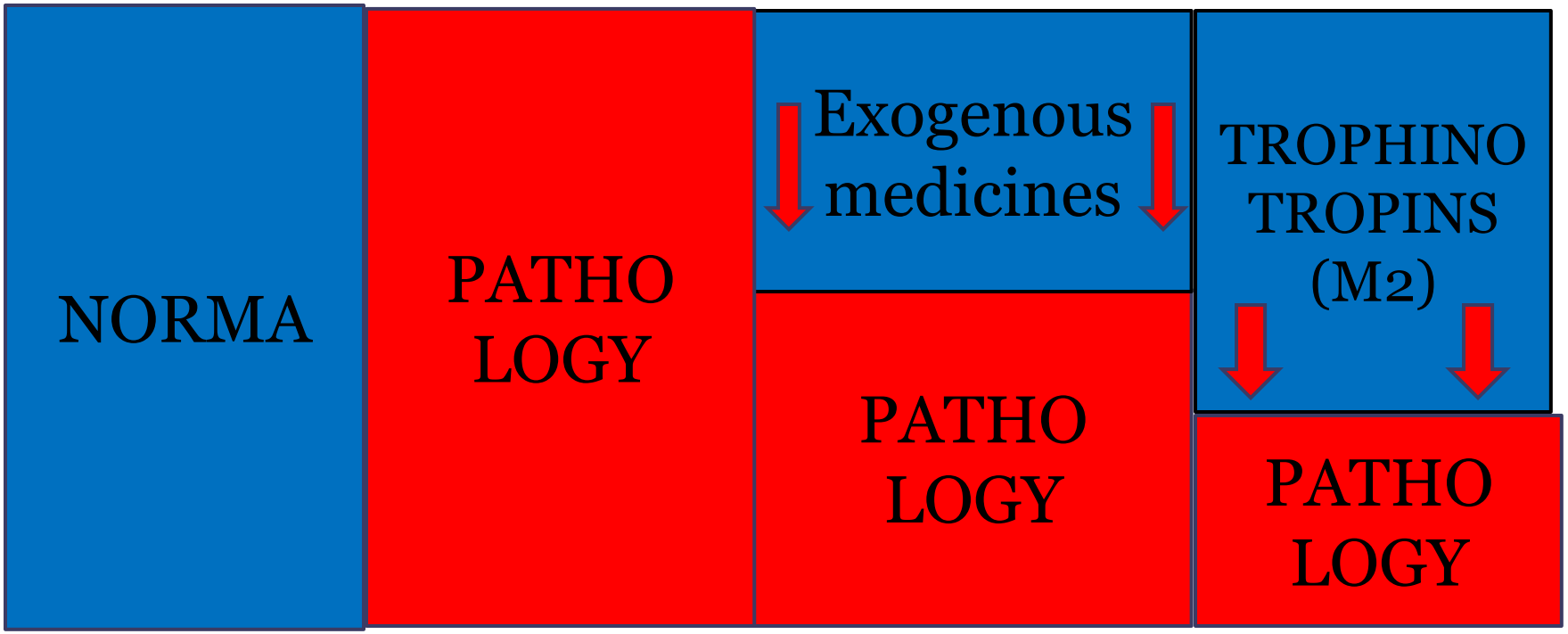
CNS cell's stages of reacting during hemorrhagic stroke modelling TTr



Peculiarities extraction from organisms tissues multipharmacological activated substances and trophitropins



Pharmacoeffectivity of TTr



MODERN DIRECTION IN PHARMACOLOGY

SYNTETIC, EXOGENIUS

- Sulindac
- DETC-MeSo
- Progesteron

PRODUCTS FROM INTACT TISSUES

- Cerebrocortex (Cortexin)
- Brain (Cerebrolysin)
- Blood (Actovegin)
- Other tissues:
  1.Kolimak
  2.Dynormin
  3.Defensin

PRODUCTS FROM RECOVERED TISSUES (TROPHINITROPINS)

- Cerebral
- M2 (Mitocorrectin)
- Postrong

Multipharma-cological action

- GCSF
- ACTH

PRODUCTS FROM AFFECTED TISSUES

- “Activated” serum (Burda I., 2015)
- Actoinvit

PERSONALIZED

- Invital
- Adement
- Autobiotic

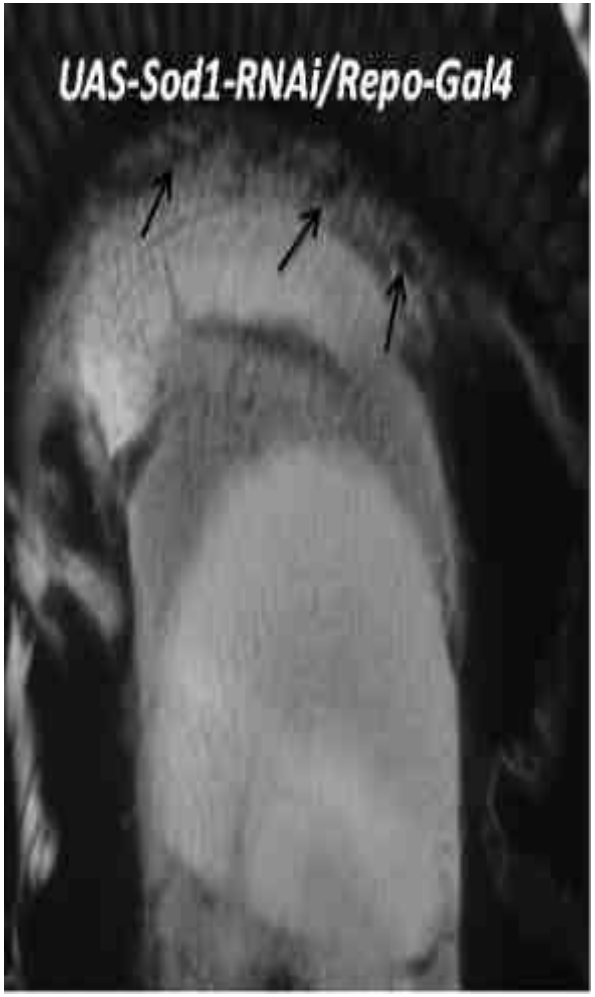
STEM CELLS

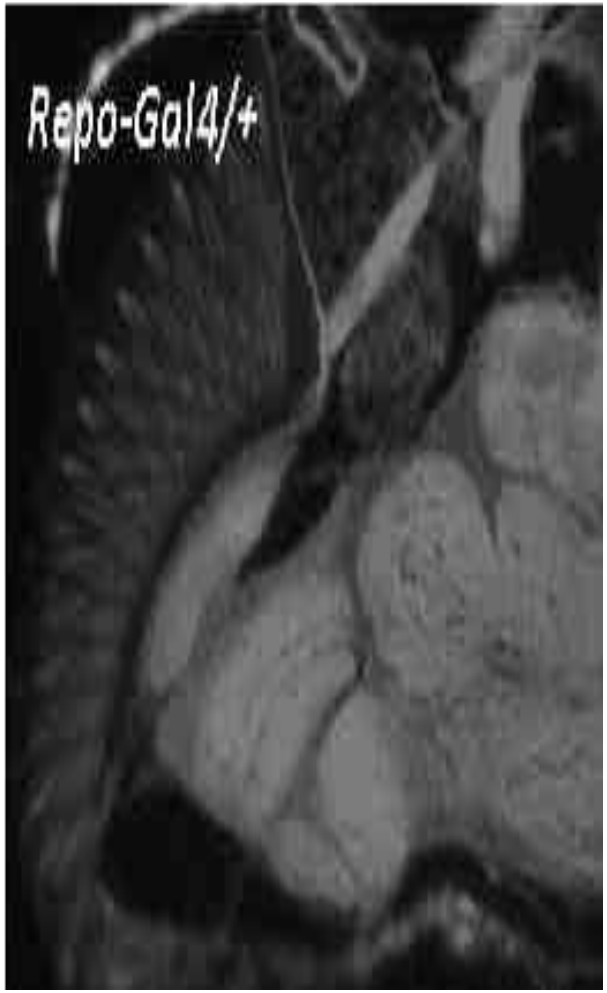
- Mesenchimal stem cells (MSC)

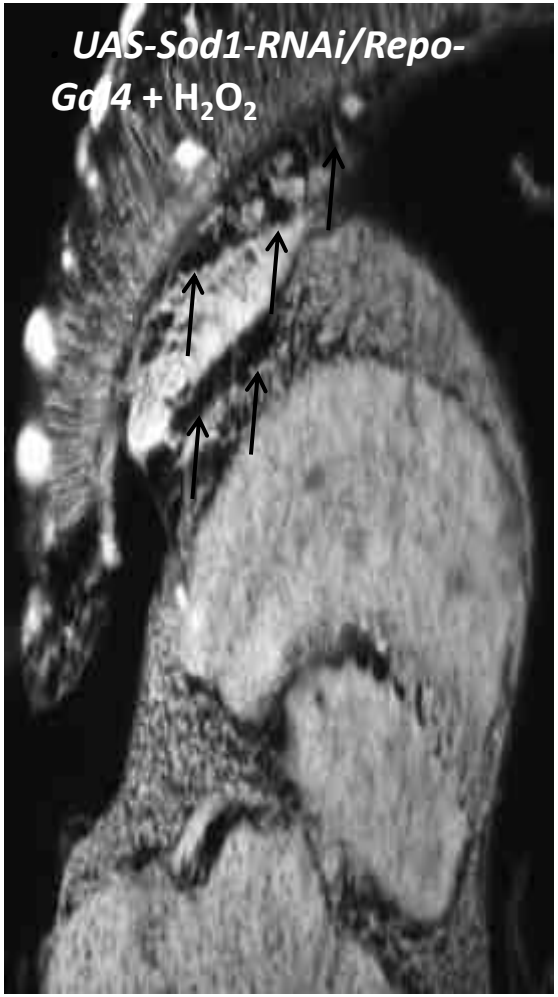
RESULTS AND DISCUSSION

- *SOD1* gene mutants is characterized by hyper wrapping of gliocytes terminals that surround neurons, vacuolization, and early die-off of neurons according to the apoptotic scenario

Total Areas of Zones of Degeneration in Brain Tissue of *D.melanogaster* Functional knockout of *Sod1*

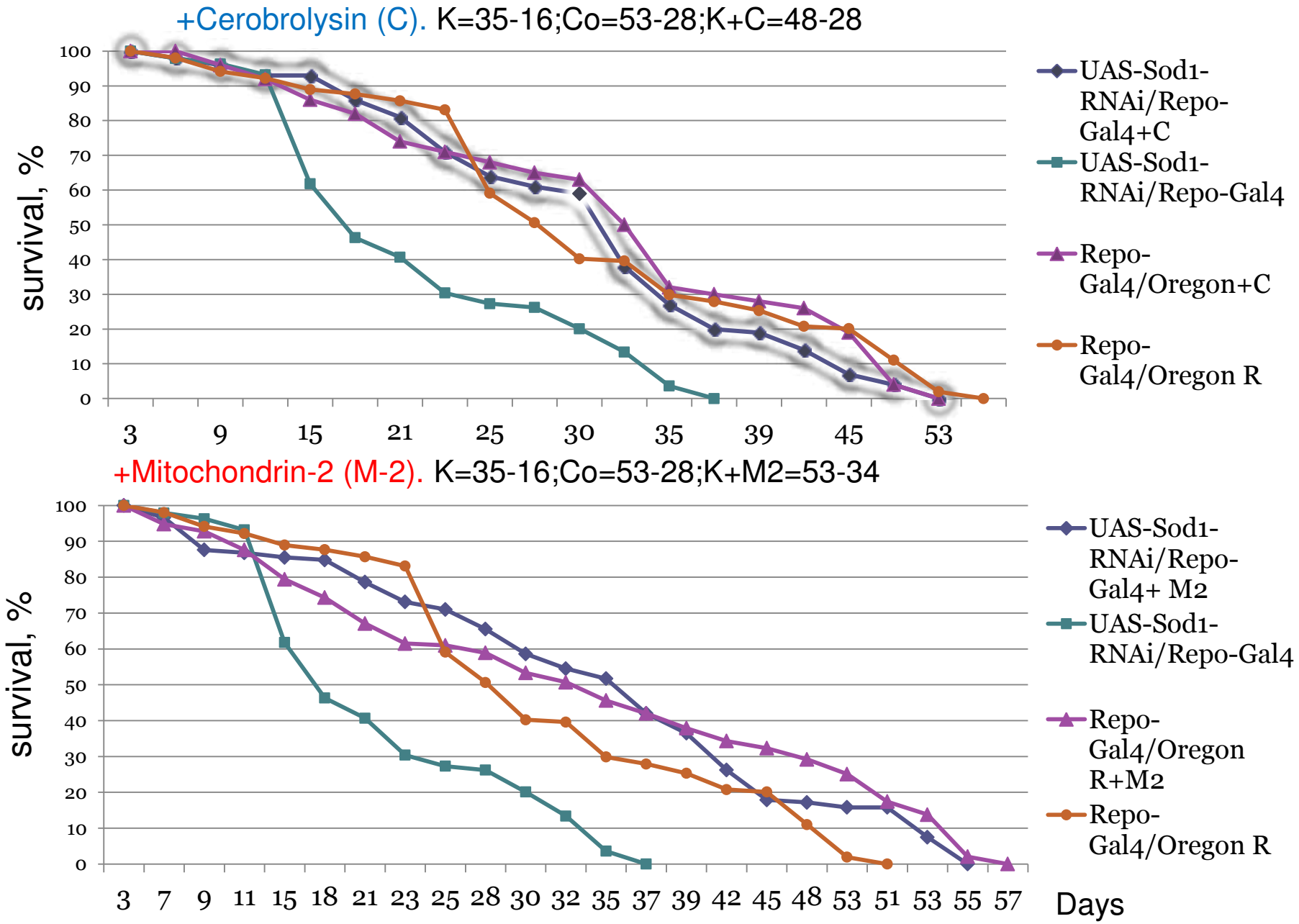






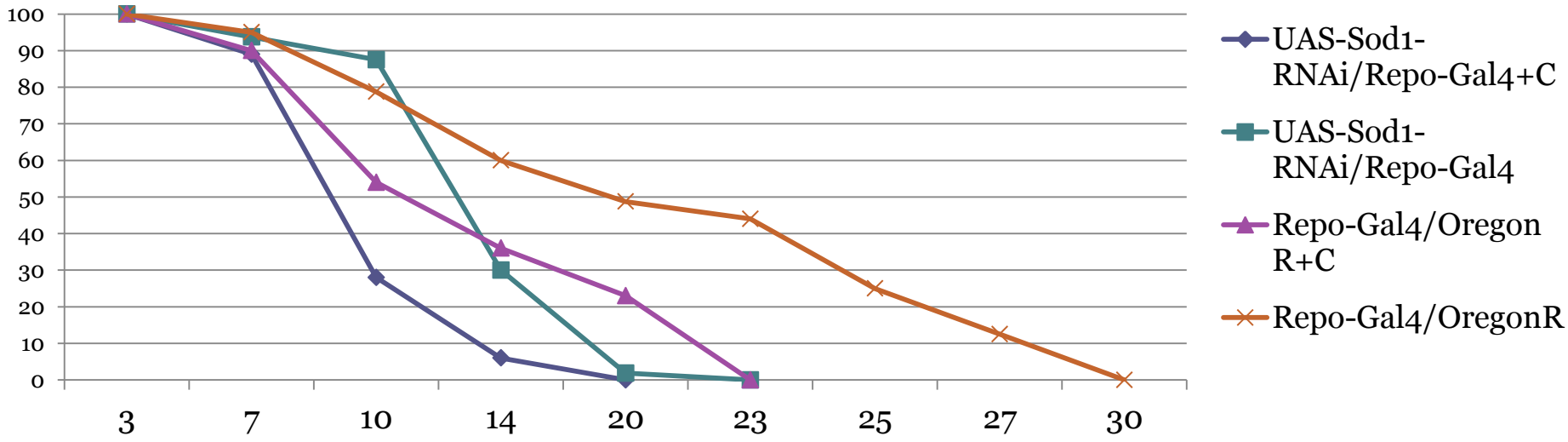
Vitushynska M., Matiytsiv N. and Chernyk Y. //Tsitologiya i Genetika. 2015.V.49, №2. P. 21-28.

SURVIVAL OF INDIVIDUALS UNDER STANDARD CONDITIONS WITHOUT AND AFTER C AND M2 TREATMENT. STANDARD MEDIUM

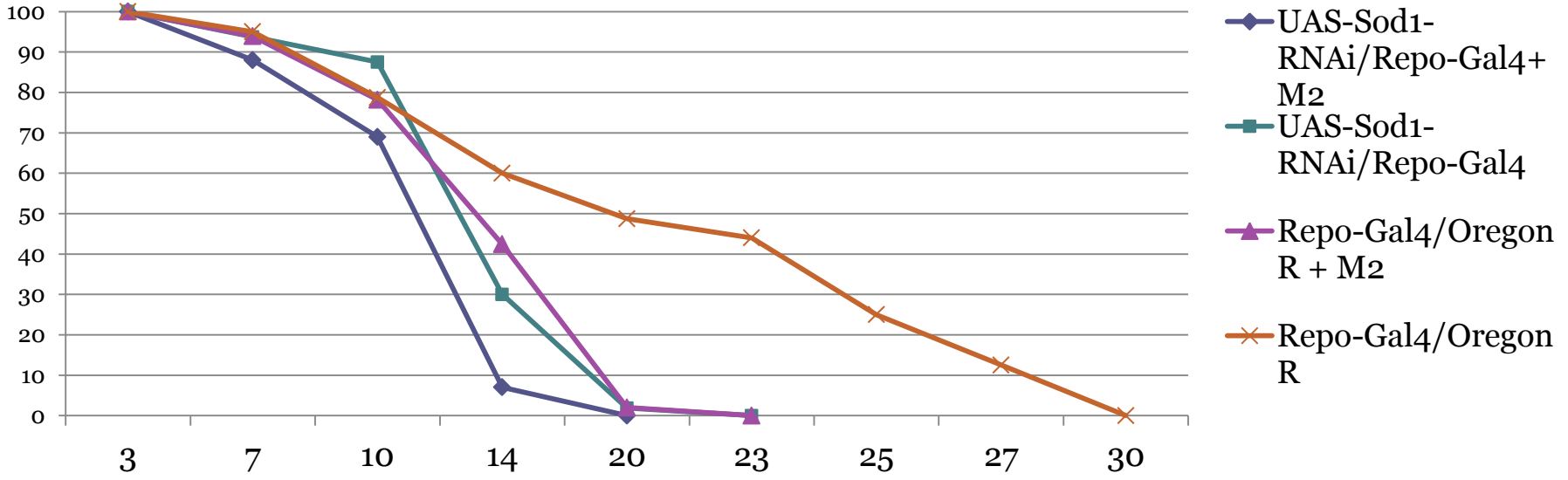


SURVIVAL OF INDIVIDUALS DURING 0XIDATIVE STRSS WITHOUT AND AFTER C AND M2 TREATMENT. 5% H_2O_2

+Cerobrolysin (C). K=23-12;Co=30-20;K+C=20-8



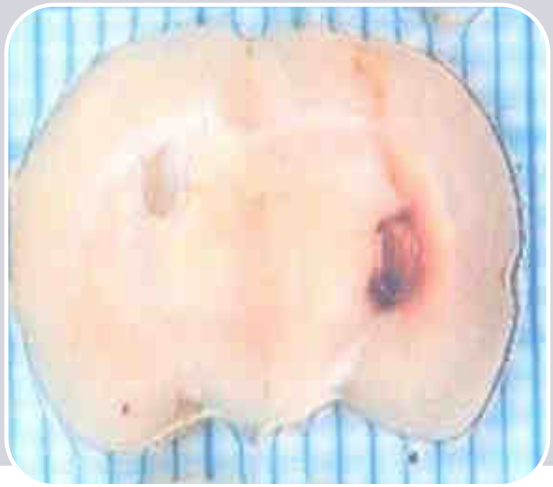
+Mitochondrin-2 (M-2). K=23-12;Co=30-20;K+C=20-14

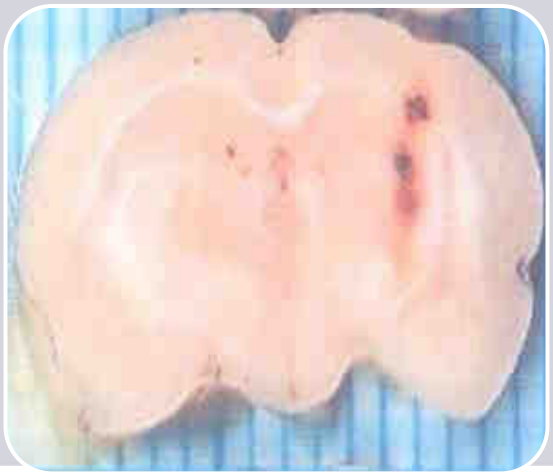


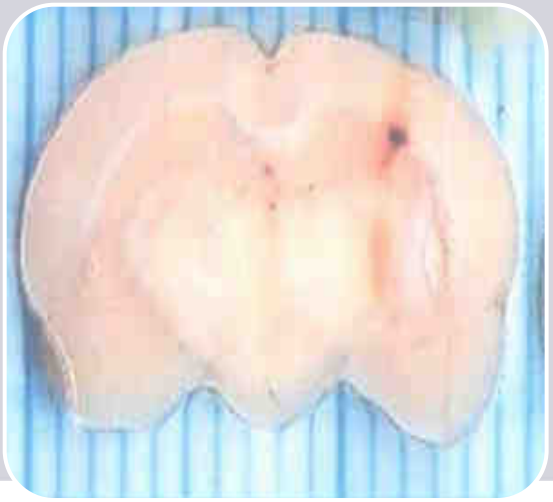
CONCLUSIONS

- Our previous data demonstrated that the tested preparation M-2 is capable of exerting certain neuroprotective effect on cerebral cells in old individuals.
- Normally individuals with Sod1 induced glial dysfunction have significantly shorter maximum life span and a low viability compared with control flies.
- Mitohondrin (M2) contains growth, trophic, antihypoxic and protective factors that have membrane-stabilizing and cytoprotective actions.
- Life span parameters become nearly to control after Mitochondrin-2 treatment.
- Mitochondrin-2 was characterized by some best values in comparison with Cerebrolysin.
- In case of oxidative stress development, the application of M-2 and C intensified their toxic effect, especially strongly this property was shown by C.

Neuronoprotective and glioprotective effect of medicines under acute HS in the experiment







Cerebrolysin
(Ever, Austria)
Neurons
III < III 3%
V < V

Gliocytes haven't any effect

Cortexin
(Geropharm, Russia)
Neurons
8% III < III
V < V 20 %
Gliocytes
III 4 % > III
Gliosis

Cerebral (TTs)
Neurons
III 8% > III 5 %
V < V 19 %

III 30 % > 12 %
V 30 % > V 8 %
Satellitosis

THANK YOU FOR YOUR ATTENTION!