Dimebon slows the progression of neurodegeneration in transgenic model of synucleinopathy

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Abstract and Introduction

A key molecular event in pathogenesis of many neurodegenerative diseases is aggregation of specific proteins followed by their deposition in characteristic inclusions within or outside of neuronal or glial cells. In most of clinical cases there is a direct correlation between the degree of aggregation estimated by the number of pathological inclusions and the degree of their spreading in the nervous system as well as the severity of pathology of the disease. Therefore protein aggregation and clearance of already formed aggregates are among the most important therapeutic targets for drugs that can effectively retard and prevent symptoms.

In this study we used a novel mouse model of synucleinopathy to determine whether a new drug (Dimebon) that was developed at our Institute ameliorates neurological pathology caused by the aggregation of proteins. As noted, in the course of our studies we developed the model for more pronounced pathological changes in the spinal cord (multiple amyloid inclusions, activation of astroglia and loss of motor neurons and their axons), even at the initial stages of the disease. Two groups of experimental transgenic animals received Dimebon (10 µg/ml in drinking water) starting at the age of 3 months, i.e. before the onset of obvious clinical signs, or 5 months when these signs are obvious. Retardation was used to assess motor neuron function and both drug-treated groups significantly improved their performance better than their control littermates that did not receive Dimebon.

Histological analysis of spinal cord sections of 12 month old transgenic animals that received Dimebon for 5 months revealed substantial decrease in number of Congo Red-positive amyloid inclusions compared to untreated animals and even to 6 month old animals. We also noted significantly reduced astroglia in the spinal cord of Dimebon-treated animals that concluded at least in this model Dimebon treatment decreases protein aggregation in the nervous system and/or accelerates clearance of already formed aggregates.

Results and Discussion

A new drug Dimebon was developed in the Institute of Physiologically Active Compound of the Russian Academy of Sciences shows neuroprotective action and may become the first pharmacological agent that modifies the development of molecular and cellular pathogenesis of proteinopathies. Using in vivo model of synucleinopathy (a transgenic mouse with the pan-neuronal synuclein overexpression) we have shown that Dimebon may reduce the number of pathological protein inclusions in neuronal cell bodies. These data provide the first evidence that Dimebon affects molecular and cellular processes of formation and/or clearance of pathological protein deposits in nervous cells. The study of direct molecular targets of Dimebon would outline the new original strategy for screening and development of a new generation of drugs for treatment of neurodegenerative disorders.

Recently, it was shown that Dimebon prevents protein aggregation in a fundamentally new model of proteinopathy which is characterized by the presence of non-amyloid fibril-like inclusions composed of TDP-43 protein. We studied the effects of methyl blue (MB) and Dimebon on formation of TDP-43 aggregates in transfected SH-SY5Y. Following treatment with 0.5 µM MB or 5 µM Dimebon, the number of aggregates was reduced by 50% and 45%, respectively (Fig. 5). The combined use of MB and Dimebon resulted in a 80% reduction in the number of TDP-43 aggregates. These findings were confirmed by western blotting analysis indicating that Dimebon may be used as a new therapeutic agent for treatments of TDP-43 related proteinopathies.

Dimebon, which was used for a long time as a non-toxic antihistaminic drug, substantially improved cognitive functions and diminished several symptoms of AD patients. Importantly, cognition and behavior of patients that received Dimebon and not other anti-dementia drugs improved not only comparing to placebo group but also comparing to their baseline conditions at the start of 26 week treatment (using five independent efficacy endpoints). Fig. 3. The beneficial effect of Dimebon over placebo was even higher after 12 month treatment period. This suggests that this drug might have a disease-modifying effect although its mechanism is not completely clear and could not be explained by previously known pharmacological properties.

Conclusions

The presynaptic overexpression of TDP-43 in mice mimics a progressive neurodegenerative phenotype, characterized by behavioral and histological features that resemble those seen in synucleinopathy models. The results obtained in this study provide evidence that the use of mouse model and AD patients chronic administration of Dimebon substantially delays the development of pathological processes induced by the increased expression of amyloidogenic proteins. These data present the first direct indication that Dimebon may affect molecular and cellular processes of formation of protein deposits and/or clearance of those pathological inclusions in various parts of the nervous system.

References


Acknowledgements and Collaborators

Work is supported by Russian Foundation for Basic Research (No. 09-04-01412-a) and the project of Russian Academy of Sciences “Fundamental Sciences for Medicine”.

Our collaborators are Prof. Y.L. Bachurin (School of Biosciences, Cardiff University, UK), Michel Godinett, MRC Laboratory of Molecular Biology (Hills Road, Cambridge, UK); Masato Hasegawa (Department of Molecular Neurobiology, Institute of Psychiatry, Tokyo, Japan) and Medicina, Inc. San Francisco, USA.

Expression of α-synuclein in Wild-type and transgenic mice

Dimebon significantly improves cognitive functions of AD patients

Expression of γ-synuclein in Wild-type and transgenic mice

Dimebon – 3,6-dimethyl-9-(2-methyl-pyridyl)-5(1H)-ethyl-1,2,3-tetrahydro-carboline dihydrochloride

Astrogliosis in the spinal cord of γ-synuclein transgenic mice

Fig. 1: Western blot analysis of α-synuclein in the spinal cord of 12 month old wild-type (Wt) and homozygous transgenic animals. The top panel shows a normalised Western blot simultaneously probed with antibodies against GAPDH and α-synuclein. This shows the fold-change in α-synuclein mRNA level increase (mean±s.e.m.) as the spinal cord of heterozygous and homozygous 8 week old symptomatic transgenic mice comparing to the level in the trigeminal ganglion of newborn wild type mice (Wt) and homozygous 12 month old symptomatic transgenic mice comparing to the level in the DRG of twelve month old Wt mice (C).

Fig. 3: Administration of Dimebon to AD patients with mild to moderate stages. The mean change in the ADAS-cog from baseline to week 52, with use of an observed case analysis. Time line shows SC 50.000 for the last 12 weeks. Retarded recovery at week 52. The horizontal line represents observed case data from only the 118 patients who passed into Phase IIa and who crossed into Phase IIIa trial. The brackets beneath the graph represent the actual number of patients with data at that visit. [Lancet. 2008; 372:205-215]

Fig. 5: Immunohistochemical analysis of the effects of methyl blue (MB) and Dimebon on the aggregation of TDP-43 in SH-SY5Y cells expressing TDP-43 (ANL.MI.187-192). TDP-43 inclusions were stained with anti-pS409/410 antibody and detected with Alexa Fluor 488-labeled secondary antibody. Representative confocal images from cells treated with DMDO (A) and 0.05 µM MB + 5 µM Dimebon (B) are shown. C is a quantitative of cells with TDP-43 aggregates. The number of cells with immunoreactive TDP-43 aggregates was counted and expressed as the percentage in the absence of treatment (taken as 100%). Time frame immunochemistry within area of approximately 900x500µm was assessed by confocal microscopy. The intensity of FITC was calculated as the ratio to nuclear staining. At least 8 areas per sample were measured (n=6-8). Data are means±S.E.M. (P<0.01 by Student’s t-test).

Fig. 7: Dimebon delays the development of locomotor and coordination disorders in ageing transgenic mice. The animals were tested on an accelerated Rotarod in 3 and 6 months after the beginning of treatment. Each column corresponds to the mean value ± SEM in rats with control transgenic (control) and homozygous transgenic (transgene) mice treated (n=9) and non-treated with Dimebon during the age of 3 months. Panel A shows results of locomotor tests for mice that received Dimebon from the age of 3 months. Panel B are mice on Dimebon from the age of 6 months.

Fig. 8: Dimebon reduces the number of amyloid deposits accumulated in the grey matter of spinal cord of ageing transgenic mice. Congo Red staining of spinal cord sections of 12 month-old transgenic (control) and homozygous transgenic mice who did not receive (B) or receive Dimebon (C) from the age of 6 months. Scale bar 25 µm.

Fig. 9: Dimebon ameliorates atrophy in the spinal cord of ageing transgenic mice. Representative pictures of the spinal cord sections of 12 month-old transgenic (control) and homozygous transgenic mice that received (transgene Dimebon) or did not receive Dimebon (transgene) from the age of 6 months. Scale bar 25 µm.