Testing of neuroprotective compounds in a transgenic mouse model of Huntington’s disease

Summary of PhD Thesis

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List of abbreviations

DA dopamine
DOPAC 3,4-dihydroxyphenylacetic acid
GABA gamma-aminobutyric acid
HAT histone acetyltransferase
HD Huntington's disease
HDAC histone deacetylase
HPLC high performance liquid chromatography
HVA homovanillic acid
KAT kynurenine aminotransferase
KYNA kynurenic acid
L-KYN L-kynurenine
MSN medium-sized spiny neuron
NMDA N-methyl-D-aspartate
PGC1-α peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PPAR-γ peroxisome proliferator-activated receptor gamma
QUIN quinolinic acid
SRS stereotypy rating scale
tg transgenic
wt wild-type
I. Introduction

Huntington’s disease (HD) is an autosomal dominantly inherited progressive neurodegenerative disorder. Amongst clinical symptoms, the motor symptoms are particularly important, because they can lead to a loss of independence in motion, resulting in a constant need of care. The pathological alterations are mainly seen in the striatum, where the loss of gamma-aminobutyric acidergic (GABAergic) medium-sized spiny neurons (MSNs) is the most pronounced feature. HD is caused by the expansion of the cytosine-adenine-guanine repeat in the gene coding for the N-terminal region of the huntingtin protein, which leads to the formation of a polyglutamine stretch.

It has been shown that the mutant huntingtin protein interacts with numerous proteins which regulate important cellular processes, thereby deteriorating their normal function. One group of these proteins is the histone acetyltransferases (HATs). The posttranslational acetylation of histones by HATs at lysine residues results in the neutralization of those positively charged proteins, leading to a more open conformation of the deoxyribonucleic acid, allowing the binding of certain transcription factors. The histone deacetylases (HDACs) remove the acetyl groups, which results in a reduced ability to bind the transcription factors, and thus the transcription processes become considerably reduced. There is increasing evidence that an altered balance between HAT and HDAC activity may accompany the development of chronic neurodegenerative disorders, including HD. The inhibition of HDACs might be able to restore the altered balance, and the application of such substances was found to mitigate the polyglutamine-dependent neurodegeneration in different models of polyglutamine disease.

Mutant huntingtin has been shown to be able to bind directly to mitochondria, thereby altering their normal function. The repression of transcription factors, e.g. that responsible for peroxisome proliferator-activated receptor gamma (PPAR-γ) coactivator 1-alpha (PGC1-α; involved in the regulation of gene expression related to mitochondrial biogenesis and respiration), are certainly associated with the mitochondrial dysfunction in HD. It seems that PPAR-γ agonists are capable of the activation of PGC1-α with the possibility of disease amelioration.

Glutamate-induced excitotoxicity seems to play an important role in the development of HD. To explain the selective impairment of the MSNs, it should be borne in mind that they
receive a massive glutamatergic input from the cortex and the thalamus. As the N-methyl-D-aspartate (NMDA) receptors are to be found in especially high amount on the spines of the MSNs, these neurons are rather sensitive to glutamate. Glutamate excitotoxicity can be influenced to a great extent by the metabolites of the kynurenine pathway, the main pathway of the tryptophan metabolism. One of the neurochemical features of HD is the imbalance of the tryptophan metabolism in the striatum in different stages of the disease. A relative decrease in kynurenic acid (KYNA) level and a reduced activity of kynurenine aminotransferase (KAT), the enzyme responsible for KYNA biosynthesis, have been demonstrated in the striatum of HD patients. These alterations have been suggested to be related to the development of the disease. Mice with the deletion of KAT-II gene displayed an increased neuronal vulnerability to the intrastriatal administration of quinolinic acid (QUIN), the possible endogenous neurotoxin in HD.

At present, only symptomatic therapy is available in HD. A number of agents have proved to slow down the neurodegeneration in different models, and have recently been tested in early-phase clinical trials. Depending on the molecular mechanism of actions, the aims mostly involve the inhibition of transcription and histone deacetylation, the achievement of marked antioxidant and antiexcitotoxic effects, improvement of the mitochondrial function and the prevention of energy impairment. Furthermore, there are also strategies to prevent the toxic effects of polyglutamine-expanded proteins. However, most of the clinical trials failed to prove any efficacy in the amelioration of disease progression.

As an animal model of HD, we used the N171-82Q transgenic (tg) mice to test compounds. Characteristically in this strain, a reduced locomotor activity and exploratory behaviour develop by aging and the pathological aggregation processes closely resemble those in human cases. Finally, the animals die at an average age of 110-130 days. This tg model of HD therefore seems to be appropriate for the testing of drug effects on survival, gait impairment and histopathological alterations.

Valproate is a widely used antiepileptic drug. It can increase brain GABA levels through the reduction of GABA-transaminase, increasing the glutamate decarboxylase activity, and it can potentiate the release of GABA. Furthermore, valproate can suppress NMDA-evoked transient depolarizations, and it can therefore exert a dose-dependent
neuroprotective effect against excitotoxicity. In addition, valproate can exert effects on the brain dopamine (DA) concentrations, too. Furthermore, it can inhibit the HDACs.

Tg mouse models of HD replicate the disturbance in the tryptophan metabolism rather well. A relative deficiency of the neuroprotective KYNA is a characteristic feature of most of these models, which raises the possibility that increasing KYNA effect is beneficial from a therapeutic aspect. KYNA can inhibit NMDA receptors at the glycine-binding sites and furthermore, it can non-competitively inhibit α7-nicotinic acetylcholine receptors, too. Blockade of these nicotinic receptors can also mediate the inhibition of glutamate release in the striatum. However, the systemic administration of KYNA does not seem a good therapeutic approach, because it has a reduced solubility, it penetrates the blood-brain barrier poorly, and it undergoes a rapid clearance from the brain and the body, mediated by organic anion transporters. To overcome these disadvantages, an amide analogue bearing a water-soluble side-chain, 2-N,N-dimethylaminoethylamine, in the amide moiety, has been synthesized. The intracerebroventricular injection of KYNA *per se* reduces exploratory activity, and induces ataxia and stereotypy in rats (mainly at higher doses), and KYNA has also been shown to be an anxiolytic. The possible changes in these aspects of behaviour following the treatment with the KYNA analogue may influence the motor performance of mice. The possible therapeutic use of the KYNA precursor L-kynurenine (L-KYN) also emerges, because it readily crosses the blood-brain barrier and it can be transformed to KYNA by the action of KATs.

L-Carnosine is a dipeptide with demonstrated antioxidant, antiglycator and metal chelator properties. Due to this complex mechanism of action, it may be capable to ameliorate the oxidative damage characteristic of HD.

Pioglitazone is a widely used thiazolidinedione antidiabetic, which has PPAR-γ activating effect.

The detrimental effects of β-sheet structure of mutant huntingtin and the consequential aggregation may be mitigated by ligands binding to the surface of this structure. Researchers at the Department of Medicinal Chemistry, University of Szeged, and those at the Protein Chemistry Research Group, Hungarian Academy of Sciences (Szeged, Hungary), set up an antiparallel β-sheet structure monomer model. The blind docking was performed on the entire surface of this glutamine40 molecule model. Accordingly, several compounds were selected
using the simulation model, which would be applicable in the amelioration of polyglutamine induced toxic effects. One of these compounds is BZS1514.

II. Aims

The aims of our studies were as follows:

(i) To study the effects of valproate, the KYNA analogue \(N-(2-N,N\text{-dimethylaminoethyl})-4\text{-oxo-}1H\text{-quinoline-2-carboxamide}\), L-KYN, L-carnosine, pioglitazone and BZS1514 on the survival of N171-82Q tg mice.

(ii) To examine the effects of those substances which significantly prolonged the survival (valproate and the KYNA analogue) on the characteristic behavioural changes (decreased spontaneous locomotor and reduced exploratory activity) in N171-82Q tg animals.

(iii) To exclude the additional effects of the treatment (valproate and the KYNA analogue) itself, \textit{i.e.} other than those due to symptom amelioration, on spontaneous locomotor and exploratory activity, using wild-type (wt) mice. We investigated the possible anxiolytic and stereotypy-inducing effects of the KYNA analogue, too.

(iv) To measure the striatal levels of dopamine (DA) and its metabolites after chronic valproate administration.

(v) To investigate thoroughly the effects of the KYNA analogue on anatomical and histopathological alterations characteristic of the N171-82Q tg mouse model of HD by means of body and brain weight analyses and unbiased design-based stereological procedures.

III. Materials and methods

\textit{III.1. Materials}

Sodium valproate, L-KYN-sulfate and L-carnosine were purchased commercially. The KYNA analogue \(N-(2-N,N\text{-dimethylaminoethyl})-4\text{-oxo-}1H\text{-quinoline-2-carboxamide}\)
hydrochloride was synthesized in the Department of Pharmaceutical Chemistry, University of Szeged. Pioglitazone hydrochloride was a kind gift of Takeda Pharmaceutical Co., Ltd. (Osaka, Japan). BZS1514 was synthesized in the Department of Medicinal Chemistry, University of Szeged (Szeged, Hungary).

III.2. Animals

N171-82Q tg mice (originally obtained from Jackson Laboratories, Bar Harbor, ME, USA) were maintained on the B6C3F1 wt background in our laboratory and genotyped by polymerase chain reaction from tail biopsies. The mice were housed under standard conditions. All animal experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC) and were approved by the local animal care committee.

III.3. Treatments and survival tests

We tested the above listed 6 agents in 5 separate experiments (the survival testing of the KYNA analogue and L-KYN were carried out in the same study). The treated tg mice received intraperitoneal injections of valproate \((n = 8; 100 \text{ mg/kg/day})\), the KYNA analogue \((n = 10; 100 \text{ mg/kg/day})\), L-KYN \((n = 10; 100 \text{ mg/kg/day})\), L-carnosine \((n = 14; 200 \text{ mg/kg/day})\), pioglitazone \((n = 10; 5 \text{ mg/kg/day in suspension})\) or BZS1514 \((n = 8; 20 \text{ mg/kg/day})\). The compounds were administered at the same time each day, 5 days per week, from 7 weeks of age. Vehicle-treated tg mice served as controls \((n = 8, n = 10, n= 10, n = 14, n = 10, n = 8, \text{accordingly})\).

III.4. Open-field test

The effects of valproate and the KYNA analogue have also been tested on low locomotor activity and exploratory behaviour in the open-field paradigm. The treatment regime was the same as in case of the survival analysis. We tested 3 groups of mice in the examinations of each compound: the control wt mice \((n = 8 \text{ in both cases})\), the control tg mice \((n = 8 \text{ in both cases})\) and valproate- \((n = 8)\) and the KYNA analogue-treated \((n = 9)\) tg mice. We examined the spontaneous locomotor activity and the exploration activity of the mice once a week, 2 h after the treatment, on the same day each week. Each mouse was placed at
the centre of a box (48 x 48 x 40 cm) and its behaviour was recorded for 5 min with the aid of Conducta 1.0 software (Experimetria Ltd., Budapest, Hungary). The ambulation distance, the duration of the lack of locomotion (local time), the duration of immobility (immobility time) and the number of rearings (rearing count) were evaluated for 9 weeks (between 7 and 15 weeks of age). After calculation of the averages for the first, second and third 3 weeks in each group, the 3 experimental groups were compared.

III.5. Testing for possible behavioural side-effects of valproate and the KYNA analogue in wt mice

To search for possible behavioural side-effects of valproate and the KYNA analogue, another population of wt B6C3F1 mice was tested ($n = 8$ in each group). In the experiments, mice received either valproate or the KYNA analogue or vehicle for 9 weeks according to the treatment regime detailed above.

First, open-field tests were performed once a week, similarly 2 h after the daily treatment, and were evaluated in the same way as in the former experiment.

Secondly, in order to examine the effects of KYNA analogue treatment on anxiety-like behaviour, we applied the elevated plus maze test in the last week of treatment 2 h after the intraperitoneal injection. The numbers of open and closed arm entries and the times spent in these arms were recorded for 5 min.

Thirdly, we applied the conventional stereotypy rating scale (SRS; scaling range: 0-6 according to the stereotypic characteristic of behaviour) method, also in the last week of treatment, 2 h after the daily injection, to examine the possible stereotypic behaviour-inducing effects of the KYNA analogue. The mice were removed from their home cages and placed individually in a clean cage for observation. They were assessed over a 30-s period and were scored in accordance with the nature of their activity. The examination was repeated on a further 2 occasions at 20-min intervals, and the scores were averaged.

III.6. High-performance liquid chromatography (HPLC) measurements

In week 9 of treatment, the valproate-treated and control wt mice were decapitated and the brains were rapidly removed and placed on an ice-cooled plate for dissection of the striatum. After dissection, the samples were stored at -70 °C until measurements. The striata
were weighed and were then manually homogenized, and the suspension (2 ml of volume) was centrifuged. From the supernatant, 250 µl was transferred to a test vial, and DA and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were analysed by reversed-phase chromatography, using an Agilent 1100 high HPLC system combined with a Model 105 electrochemical detector under isocratic conditions. The mobile phase containing sodium dihydrogenphosphate (75 mM), sodium octylsulfate (2.8 mM) and disodium ethylenediaminetetraacetate (50 µM) was supplemented with acetonitrile (10% vol/vol) and the pH was adjusted to 2.8 with phosphoric acid (85%). The mobile phase was delivered at a rate of 1 ml/min at 40 ºC onto the reversed-phase HR-80 C18 column after passage through a Hypersil ODS pre-column. Ten-microlitre aliquots were injected by the autosampler with the cooling module set at 4 ºC. The signals captured by the Model 105 electrochemical detector were converted by an Agilent 35900E dual-channel interface and the chromatograms were evaluated by ChemStation Rev.1.10.02 software.

### III.7. Anatomy and immunohistochemistry

The body weight of the mice, treated with the KYNA analogue, was measured once a week on the same day each week, at the same time of the day, from 7 weeks of age. When the animals reached 16 weeks of age, they were deeply anesthetized and perfused transcardially with 0.9% NaCl solution, followed by 4% paraformaldehyde. The perfused brains were removed, weighed and post-fixed with 4% paraformaldehyde for 24 h. The whole striatum (n = 7 in each group) was sectioned on a vibratome at 60-µm thickness and the sections with a distance of 420 µm (between the upper surfaces) were collected in a systematic random fashion in 7 vials. The sections were cryoprotected, and after freeze–thawing, the sections of the left hemisphere were washed repeatedly, and were processed for immunostaining using either anti-NeuN, or anti-huntingtin primary antibodies and thereafter biotinylated donkey anti-mouse secondary antibody, followed by avidin-biotinylated horseradish peroxidase complex. The immunoperoxidase reaction was developed by using 3,3’-diaminobenzidine as a chromogen. The sections were treated with osmium tetroxide, then dehydrated in an ascending alcohol series and acetonitrile, and embedded in Durcupan.
III.8. Stereology

We used the unbiased design-based stereology method on the Durcupan-embedded sections to estimate the number and size of the neurons in the striatum. The contours of a striatum were traced in each serial section \((n = 8-9)\), using a 10x objective of a Zeiss Axioskop 2 and the Stereo Investigator software and the striatal volume was calculated for each brain. Subsequently, we utilized a 100x objective with oil immersion for quantification of the anti-NeuN-stained neurons and anti-huntingtin-stained nuclei with the optical fractionator method. Furthermore, the nucleator probe was utilized in our samples to estimate neuronal volumes.

III.9. Statistics

All statistical analyses were performed with the help of the SPSS Statistics 17.0 software. Survival data were analyzed by using Kaplan-Meier survival curves and the Mantel-Cox log rank test. In the cases of behavioural tests, HPLC measurements and anatomical analyses, we first checked the distribution of data populations with the Shapiro-Wilk W test, and also performed the Levene test for the analysis of the homogeneity of variances. If the data showed a non-Gaussian distribution, or the equal variances were not assumed, we used nonparametric statistics: the Mann-Whitney U test when 2 groups were compared and the Kruskal-Wallis test when there were more than 2 groups. If the data were distributed normally and equal variances were assumed, we used parametric statistics: 2 groups were compared via the independent t test and multiple groups of data were compared by using repeated or one-way measures of analysis of variance followed by Fischer’s least significant difference post hoc test comparison. The null hypothesis was rejected when the \(p\) level was < 0.05, and in such cases the differences were considered significant.

IV. Results

IV.1. Survival

Valproate, significantly increased the mean survival time of the tg mice by 31.4% \([p < 0.05]\). Similarly, the KYNA analogue also significantly increased the mean survival time of
the tg mice by 30.7% [$p < 0.05$]. In contrast, L-KYN, L-carnosine, pioglitazone and BZS1514 did not prolong the survival of tg mice.

**IV.2. Behavioural tests**

We first assessed the locomotor activity of the valproate- and the KYNA analogue-treated tg mice together with their corresponding control wt mice and tg mice by measuring their ambulation distance, local time and immobility time in the open-field during the test period for 9 consecutive weeks. During the second 3 weeks, valproate significantly increased the ambulation distance [$F_{(2,20)} = 8.716, p < 0.01$; *post hoc* test: $p < 0.001$] and significantly decreased the local time [$F_{(2,20)} = 11.63, p < 0.001$; *post hoc* test: $p < 0.001$] of the tg mice as compared with those of the untreated tg animals. During the third 3 weeks, the differences in ambulation distance were no longer significant in the experiment assessing the effects of valproate, probably due to an increase in the standard deviation. However, the significant differences in local time [$F_{(2,20)} = 15.71, p < 0.001$; *post hoc* test: $p < 0.001$] still remained between the valproate-treated and untreated tg animals. During the third 3 weeks, the treatment with the KYNA analogue restored the locomotor activity of the treated tg mice to the wt level and their performance became significantly different from that of the non-treated tg mice group both in view of ambulation distance [$F_{(2,20)} = 5.217, p < 0.05$; *post hoc* test: $p < 0.01$], and that of immobility time [$F_{(2,20)} = 3.916, p < 0.05$; *post hoc* test: $p < 0.05$]. In the independent control experiments, neither valproate nor the KYNA analogue themselves influenced the spontaneous locomotor activity of the wt mice.

We found that the treatment with valproate significantly increased the number of rearings [$F_{(2,21)} = 5.005, p < 0.05$; *post hoc* test: $p < 0.05$], which increase was still present during the whole examination period. The KYNA analogue did not change this aspect of behaviour significantly, although the difference between the treated and the non-treated mice did become larger by the end of the third 3 weeks. In the independent control experiments, neither valproate nor the KYNA analogue themselves influenced the exploratory activity of the wt mice.

The results of the elevated plus maze test showed that there was no significant difference between the KYNA analogue-treated and untreated groups as concerns the percentage of time spent in the closed arms or the number of closed arm entries. The results
on the SRS demonstrated that the treatment with the KYNA analogue did not induce the stereotypic behaviour in the wt mice.

**IV.3. HPLC measurements**

Valproate did not induce significant changes in the striatal DA, DOPAC or HVA levels at the applied dose following its chronic administration.

**IV.4. Body weight and neuroanatomical changes**

The average body weight of the wt control animals increased continuously until around 14 weeks of age, whereas no increase in body weight was detected in case of the tg mice after the age of 7 weeks, and hence the weights of the 2 groups were significantly different \[F(9,108) = 20.27, p < 0.001\]. Our results revealed that the treatment with the KYNA analogue increased the body weight of the tg mice significantly \[F(9,108) = 3.961 p < 0.001\].

With regard to the CNS pathology, the brain weight of 16-week-old tg animals was significantly lower, by 7.9%, than that of the wt mice \[p < 0.05\], but that of the treated tg animals was not significantly different. The stereologically measured striatal volume was also significantly lower \[by 12.6%; p < 0.05\] in the tg animals than in the wt mice. When the tg mice were treated with the KYNA analogue, their striatal volume was no longer different from that of the wt mice.

Unbiased stereological methods did not indicate any significant difference in the number of neurons in the striatum of the control wt mice, the tg mice or the KYNA analogue-treated tg mice. However, we found that the average size of the neurons in the striatum of the tg animals was lower by 14% \[p < 0.01\] than that for the wt mice. The treatment with the KYNA analogue completely prevented the development of atrophy of the striatal neurons in the tg mice, and the average size of these neurons was significantly different from that in the control non-treated tg animals \[\chi^2 = 13.01, df = 2, p < 0.01; post hoc test: p < 0.05\].

The treatment with the KYNA analogue did not induce a significant change in the percentage of anti-huntingtin immunoreactive (EM48\(^{+}\)) neurons in the striatum of the tg animals relative to the control tg animals.
V. Discussion

Although it is known that a definite single mutation leads to the development of HD (the best example of trinucleotide repeat expansion disorders), it is unclear how this mutation actually results in disease pathogenesis. The pathomechanism must therefore be thoroughly explored, and effective new drugs with higher therapeutic value must be developed. Thus, the main aim of the research work directly related to this thesis was to find compounds, both well-known (with the possibility of a second medical use) and newly synthesized (with innovative purposes) agents of therapeutic potential in this devastating illness.

Using the N171-82Q tg mouse model of HD, we tested the effects of valproate and the novel KYNA amide on survival. Valproate and the KYNA amide increased the survival time of the tg mice significantly (by 31.4% and 30.7%, respectively). These elevations are noteworthy: the most effective agent in this model, probenecid, lengthened the survival by 35%, while tiagabine did so by 26% and phenylbutyrate by 23%.

The beneficial effect of valproate and the KYNA amide is supported by our findings in the open-field tests, too. As regards the spontaneous locomotor activity, both agents have exerted a significant protective effect. In addition to the beneficial effects on spontaneous locomotor activity, valproate exerted a significant amelioration in the exploratory activity, too. As valproate and the KYNA analogue themselves did not influence the locomotor activity in the wt mice, and the KYNA analogue did not induce anxiolysis or stereotypy in the administered dose, the beneficial change in hypolocomotion is not due to any behavioural side-effect of these substances.

It is also worth mentioning that chronic valproate treatment did not influence the striatal levels of DA and its metabolites, which would also have influenced the motor performance of mice.

As neuroprotective properties of valproate are well supported by the histology data from the scientific literature, only preliminary qualitative examinations has been carried out on brain pathology by means of cresyl violet staining and anti-huntingtin immunohistochemistry. The findings indicate that the cellular atrophy was present in treated animals, but its extent was lower than that of untreated tg mice. These findings are consistent with the data in studies with other HDAC inhibitors, but valproate does not seem to exert such pronounced effects as those substances. Contrary to the previous studies with HDAC inhibitor
molecules, where the huntingtin aggregate deposition was not altered, our provisory data suggest that valproate treatment diminished the striatal anti-huntingtin immunoreactivity (examined with EM48 antibody). This phenomenon might be explained in that the pharmacological activity of valproate is more complex than its effects can be interpreted solely upon its HDAC inhibitor activity.

As neuroprotective properties of the novel KYNA analogue has never been verified before, thorough examinations on anatomical changes has been carried out. The body weight data well support the findings of survival and behavioural analyses, for the treatment significantly mitigated the characteristic phenomenon that the body weight of the N171-82Q tg mice does not increase further after 2 months of age. Furthermore, our brain pathology data are in accordance with the findings of Schilling et al., 1999 (the first characterization of N171-82Q tg mice), who reported that the brains of tg animals are slightly smaller (by 7.9% in our experiment). In parallel with the difference in brain weights, the volume of the striatum was lower (by 12.6%) in the N171-82Q tg animals. This phenomenon can be well explained by the 14% lower neuronal volume. In our experiments with the novel KYNA amide, the neuronal atrophy was nearly completely mitigated. As concerns the aggregation pathology, we investigated the percentage of EM48+ nuclei in the striatum. This revealed that the treatment led to a slight, but not significant improvement.

The lack of significant effects on survival in the case of L-KYN could be for several reasons. Although L-KYN has been found to be protective alone in brain ischaemia models or in combination therapy with probenecid in a migraine or ischaemia model, these beneficial effects were seen after an acute event with sudden onset. Probably the transformation of L-KYN in these acute conditions is mediated mainly toward KYNA production. However, in the N171-82Q tg HD model with prolonged disease onset and a probable disturbance of the tryptophan metabolism, L-KYN probably transforms toward the toxic metabolites, too, following its chronic administration. So although L-KYN administration seems beneficial in acute conditions, it is probably invaluable in the treatment of conditions with prolonged onset.

Although in higher doses L-carnosine has been found to be protective against permanent cerebral ischaemia in mice, in our experiments, it did not exert any significant prolongation in survival. The possible amelioration achieved by extremely high doses would have only scientific, but not therapeutic significance, because in that case the possibility of
cost-effective production and finding an appropriate route of daily administration for human use is questionable.

The phenomenon that pioglitazone has been found to be ineffective could be due to that the chronic intraperitoneal administration of suspended particles has slight local toxic effects, which although do not exert any seeming deterioration in wt animals, probably can cause disease worsening in mice predestinated to chronic progressive disease development. Furthermore, higher doses have been applied in most of the previous experiments, so subdosing would be another reason for the lack of efficacy.

The BZS1514 was previously found to be protective in a tg mouse model of Alzheimer’s disease, but it did not exert any significant amelioration of survival at the twofold dose in our experiments. The lack of clear efficacy may be explained in that the achievement of significant intracellular/intranuclear effects needs even higher doses.

Based upon our results, it can be concluded that it is worth considering the design of further preclinical studies and new clinical trials with valproate. Additionally, the novel KYNA amide also seems to be a promising candidate for trials. Although L-KYN, L-carnosine, pioglitazone and BZS1514 did not exert any significant amelioration in the survival analyses of our experiments, new dosing and administration regimes or the application of combination therapies may lead to the achievement of beneficial effects, if they have any.

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VII. Publications directly related to the PhD thesis


Total impact factor of original papers, directly related to the thesis: 5.226

Publications not directly related to the thesis


Cumulative impact factor: 28.959