
Check for updates

treatment strategies, besides those already de-
-secretase inhibitors or anti-Aβ-immunisation, is
use none of these were successful in clinical trials
2015). To meet this need, we have developed D-en-
des, solely consisting of D-enantiomeric amino acid
ypothesized mode of action of those peptides is to
dy existing toxic Aβ oligomers, which are postulated to
e and neuronal loss, by a ligand mediated stabilisation of
rs in an aggregation-incompetent, amorphous conforma-
is, the equilibrium between different Aβ species is shifted
toxic Aβ oligomers towards Aβ monomers. Thus, there is no
to rely on components of the immune system (e.g. for anti-Aβ-
isation) to remove Aβ oligomers and presumably no adverse
eaction as a consequence of a negative activation of the immune
m. This mode of action was already described for the lead com-
nd "D3". D3 eliminates toxic Aβ oligomers, as demonstrated by the
β-QIAD (quantitative determination of interference with the Aβ ag-
gregate size distribution) assay. The Aβ-QIAD assay allows analysing a
potential reduction of Aβ oligomers in vitro (Brener et al. 2015).
Moreover, D3 improves cognitive impairment, and reduces Aβ plaque
load in different AD mouse models in vivo, even after oral adminis-
tration (Brener et al. 2015; Funke et al. 2010; van Groen et al. 2013;
van Groen et al. 2009; van Groen et al., 2008, 2012). Based on a ra-
tional design of D3, the compound RD2 has been developed in order to
enhance the Aβ oligomer elimination efficacy. RD2 reveals enhanced
Aβ oligomer elimination efficacy compared to D3, which has been
confirmed in vitro (van Groen et al. 2017). Furthermore, RD2 has al-
ready proven its in vivo efficacy to ameliorate cognitive deficits in
different AD mouse models in different laboratories. In a first treatment
study, RD2 improved cognitive deficits in seven months old APP/PS1
mice during the fourth week of a four-week-treatment study via in-
traperitoneal administration (van Groen et al. 2017). In accordance to
the promising pharmacokinetic profile of RD2, and its high oral bioa-
vailability (Leithold et al. 2016), we carried out several oral treatment
studies with RD2. We treated eight months old APP_{SL} mice and found
significant cognitive improvement compared to placebo treated mice
after seven weeks of oral treatment (Kutzsche et al. 2017). In the next
study, we orally treated old-aged APP/PS1 mice with severe cognitive
deficits and full-blown AD-pathology, resulting in a reversal of cogni-
tive deficits and representing a clearly curative rather than a merely
preventive efficacy of RD2 (Schemmert et al. 2018).

Based on the ability of RD2 to reduce $Aβ_{(1–42)}$ oligomers and to
improve cognitive deficits, we hypothesized that RD2 is able to rescue
synapses and neurons from $Aβ_{(1–42)}$ and also pEAβ induced toxicity.
Therefore, we chose a mouse model with a clear neurodegeneration due
to intraneuronal accumulation of neurotoxic $pEAβ_{(3–42)}$, the TBA2.1
mouse model, to test the efficacy of RD2. Homozygous TBA2.1 mice
develop severe motor deficits and
CA1 region of th
typ
neration, especially in the
rly onset of the pheno-
gression (Alexandru
n et al. 2018b).

from this, we conf
were caused by
as in their non

ethods

nination

Binding affinity of R
plasmon resonance (SP
before (Dunkelmann e
from 0.41 μM to 300

2.2. IC_{50} determin

Inhibitory f
sessed using
pressed and
solved in H
lyophilized
20 mM so
5 μM Th
tions ra
bindin
The fl
and λ
Germ
time
by
we
Th
(G

2

study.

all mice were housed with maximal four mice per environment on a light/dark cycle (12/12 h), with d a temperature of 22 °C. Food and water were um.

s synthesized, purified and lyophilized with acetate as by CBL Patras (Patras, Greece).The amino acid sequence is H-ptlhthnrrrrr-NH2. The development and specificity of RD2 ribed previously (van Groen et al. 2017).

reatment

Non-transgenic littermates and homozygous TBA2.1 mice were eated orally for twelve weeks (daily) either with placebo ($n = 8$) (drinking water), 20 mg/kg RD2 (n = 8) or 100 mg/kg RD2 (n = 8), all formulated in tailor-made gelatine-jellies (30% sucrose, 10% sucralose, 18.75% instant gelatine (Dr. Oetker, Bielefeld, Germany), total volume approximately 200 µl with 50 µl compound or placebo solution). The RD2 amount in the jellies was weekly adjusted to the average body weight of the mice to achieve as close as possible a daily dose of 20 or 100 mg/kg. For example, each jelly for the 20 mg/kg/day RD2 treatment group contained 0.4 mg RD2 in 50 µl in the last week of treatment. The mice ate each single jelly completely and voluntarily. Thus, it was ensured that each mouse had incorporated the appropriate amount of RD2 daily over twelve weeks. In detail: the mice were taken out of their home cages each morning and were single placed in a clean cage without bedding. A jelly (either with - or for the placebo without RD2) was placed in the middle of the cage. After the mice had eaten the jellies completely, usually within a few minutes, they were placed back into the home cages. This procedure was repeated each day for twelve weeks.

2.7. Behavioural assessments

Each behavioural test was performed before treatment (baseline measurements and to randomise the mice), after six weeks of treatment (middle) and within the last week (after) of the twelve-week treatment period. In each test, all groups of homozygous TBA2.1 mice, and non-transgenic littermates were tested. Mice were inspected daily and body weight of each mouse was recorded weekly.

2.7.1. Open field test

By performance of an open field test it is possible to evaluate the anxiety, locomotive and explorative behaviour of mice. Therefore, mice were placed in a square-shaped m × 44 cm × 44 min). For evaluation, the into two departments: cent er: 44 cm × 44 cm). in. Recording was

Z treatment comp
llowing subtests
ness, gait, start
ex, cornea refl
n response. P
m (0: no ab
s used for

2.7.3. Clasping test

Analysis of the inna
the mice up by the tail
limbs was observed
abnormalities). Th
and the sum of al

2.7.4. Pole test

In this stu
was used to
Distinguishe
downward
meter 1.2
was rate
run, 2:
formed
sum o

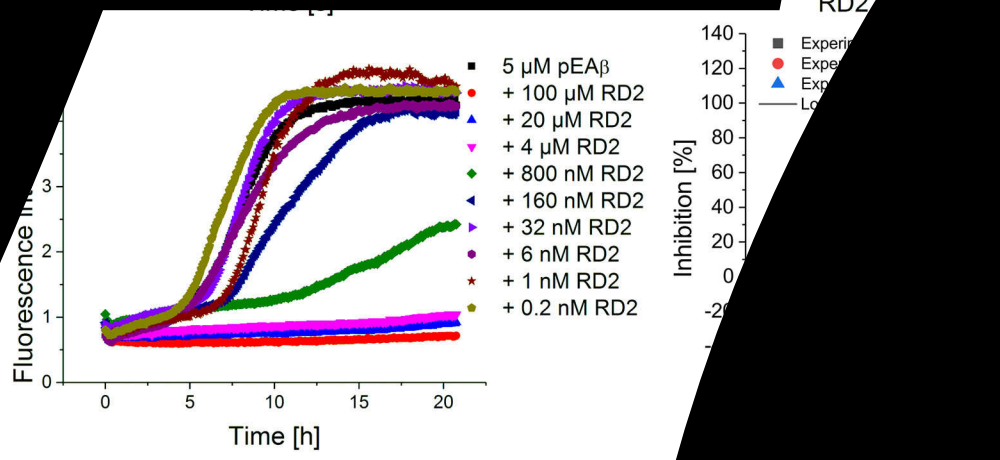
2.7.5

N
TBA
ma
Co
et
o
s
n
a
l

2

2.

fr
RD
tem
low
acid



g. 1. Binding affinity of RD2 to pEAβ$_{(3–42)}$ and inhibitory efficacy of RD2 on pEAβ$_{(3–42)}$ fibr
different RD2 concentrations binding to immobilized pEAβ$_{(3–42)}$ (A). Equilibrium dissocia
against applied RD2 concentrations and fitted using a Langmuir 1:1 binding model (B). Sho
mean ± SD of three independent experiments. Fibril formation of 5 µM pEAβ$_{(3–42)}$ in t
Thioflavin T over 21 h (C). Final fibril masses at the end of this time period were normaliz
maximal inhibitory concentration (IC$_{50}$, 707 nm and a Hill coefficient of 3) was determine
of three replicates and three independent experiments. The IC$_{50}$ value is presented as n

incubation in 3% H_2O_2 in methanol (15 min). In between these steps, the sections were washed three times with 1% Triton in TBS (TBST) for 5 min, respectively. Incubation with the primary antibodies (6E10: 1:2500, Bio Legend, San Diego, USA; GFAP: 1:1000, DAKO, Agilent Technologies, Santa Clara, USA, NeuN, 1:1000, Merck, Germany; CD11b 1:2500, Abcam, UK) diluted in TBST with 1% bovine serum albumin (BSA) was performed in a humid chamber at 4 °C over night. The next day, incubation with the biotinylated secondary anti-mouse- or anti-rabbit-antibody (both 1:1000 in TBST + 1% BSA, Sigma-Aldrich, Darmstadt, Germany) was performed at room temperature for 2 h. Staining was visualized by use of 3, 3′-Diaminobenzidine, enhanced with saturated nickel ammonium sulphate solution. Finally, the sections were washed in an ascending alcohol series and mounted with DPX Mountant (Sigma-Aldrich, Darmstadt, Germany).

2.9.2. Quantification

All slides were stained in one staining procedure and microscope recordings were done in one microscope session to avoid differences in staining intensity and light exposure. A Zeiss SteREO Lumar V12 microscope and the according software (Zeiss AxioVision 6.4 RE) or a Leica LMD6000 microscope (LAS 4.0 software) were used for recording. Quantification was performed with ImageJ (National Institute of Health, Bethesda, USA). Aβ partic analysed in the striatum, motor cortex, hi midbrain (inferior colli-culus 2 20 mg n = 8, RD2 stained area) was

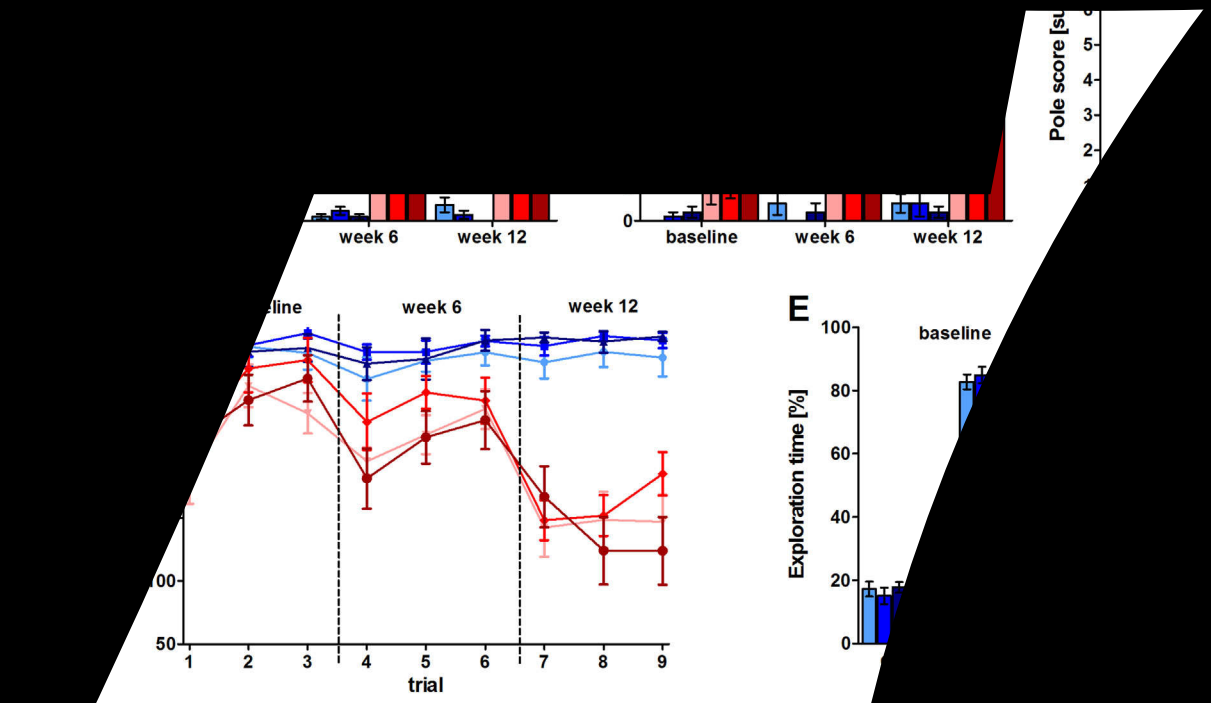


Fig. 2. Evaluation of motor, exploratory and anxiety related behaviour of RD2-treated hom (ntg). Investigation of an amelioration of the phenotype of homozygous TBA2.1 mice conducted by different behavioural test. Analysis of the SHIRPA test battery (A), clasping the phenotype of both RD2 treatment groups compared to placebo-treated mice. Addi investigate possible adverse drug reactions on motor related behaviour. In each test, Analysis of the rotarod test did not result in a significant amelioration of the motor imp concentration (D). No effect regarding a negative influence of RD2 treatment on ntg w after six, and after twelve weeks of treatment. Each given trial in (D) represents three decreased exploration or anxiety related behaviour of ntg or hom mice, either treated has no adverse effect on anxiety or exploration. Data is represented as mean ± SEM. S hoc analysis, $n = 8$ for all groups and each test. $^{*}p = .05$, $^{**}p = .01$, $^{***}p = .001$.

in liquid nitrogen and stored at −80 °C until further processing. The pyroglutamate Aβ ELISA N3pE-42 was purchased from IBL (IBL International GmbH, Germany) and the $A\beta_{(x\text{-}42)}$ ELISA was purchased from BioLegend (BioLegend, San Diego, CA, USA), and performed according to the manufacturer's protocol with the three fractions of all treatment groups (placebo $n = 8$, RD2 20 mg n = 8, RD2 100 mg n = 8) described above and each sample was measured in triplicate.

2.10. Statistical calculations

Descriptive statistical analysis was performed on all evaluated parameters. Data is represented as the average mean ± SD (in vitro), or SEM (in vivo). All calculations were performed by use of OriginPro 8.5G (OriginLab, USA), or SigmaPlot Version 11 (Systat Software, Germany), $p > .05$ was considered as not significant (n.s.). Normal distributed data was analysed with one-way ANOVA with Fisher post hoc analysis. Two-way or two-way repeated measure (RM) ANOVA with Fisher post h lyse parts of the in vivo study

f treatment) to test the efficacy of RD2 with dif- on phenotype progression of homozygous TBA2.1 ic littermates were tested in parallel to assess spe- tment and putative side effects. All mice were mon- cause of the severe phenotype of the used mouse

ent did not cause any loss or gain of body weight in the c or homozygous mice in comparison to placebo treat- ective of the administered RD2 concentration. Moreover, no changes in the general physiological conditions of the bserved by daily visual inspection and weekly body-weight g. Also, macroscopic investigations at the end of the experi- id not reveal abnormalities of any organ of RD2-treated non- genic littermates or homozygous mice, even in the group which treated with 100 mg/kg RD2 daily.

To evaluate, whether RD2 has any influence on the progression of e neurodegenerative phenotype of homozygous TBA2.1 mice, or any mpact on non-transgenic littermates, different behavioural approaches were conducted (Fig. 2).

By use of the SHIRPA test battery, it was tested whether there was a difference in the neurodegenerative phenotype of homozygous TBA2.1 mice, treated with placebo or different concentrations of RD2. At the end of the treatment period (after twelve weeks of treatment), both RD2 treatment groups were scored with significantly lower SHIRPA scores compared to placebo-treated mice (two-way RM ANOVA, $F_{(5,84)} = 54.27$, $p < .001$, Fisher LSD post hoc analysis placebo vs. 20 mg/kg/day $p < .001$, placebo vs. 100 mg/kg/day $p < .001$, 20 mg/kg/day vs. 100 mg/kg/day n.s. $p = .95$, Fig. 2 A). Moreover, daily treatment with either 20 mg/kg or 100 mg/kg RD2 slowed down the phenotype progression measured in the SHIRPA test compared to placebo-treated mice during the second half of the treatment period (two-way RM ANOVA, $F_{(5,84)} = 95.70$, $p < .001$, Fisher LSD post hoc analysis week six vs. week twelve of treatment placebo $p < .001$, 20 mg/kg/day $p = .002$, 100 mg/kg/day $p = .018$, Fig. 3 A). At each tested time point, RD2- or placebo-treated non-transgenic littermates showed no abnormalities in behaviour. They revealed SHIRPA scores around zero, indicating no change of any evaluated parameter due to treatment with different concentrations of RD2 (Fig. 2 A).

By use of the clasping test, the hind limbs of the mice were analysed (Fig. 2 B). A considerable decline in the clasping behaviour of homozygous mice (increasing clasping scores) was observed during the course of the treatment period. Although clasping behaviour of homozygous RD2-treated mice deteriorated as well, both RD2-treated groups showed significantly lower clasping scores compared to the homozygous placebo-treated group at the end of the treatment period (two-way RM ANOVA, $F_{(5,84)} = 77.05$, $p < .001$, Fisher LSD post hoc analysis placebo vs. 20 mg/kg/day $p < .001$, placebo vs. 100 mg/kg/day $p = .002$, 20 mg/kg/day vs. 100 n.s. $p = .5$, Fig. 2 B). No change in the cl nsgenic littermates was obse

otor deficits was

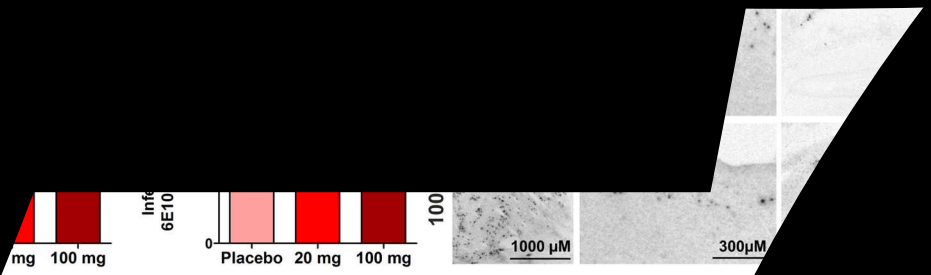
ble test resulted i bo-treated homo (two-way RM A analysis week placebo vs. 1 /day n.s. p ssion of t zygous TBA2.1 mice, but n sion was measureable wit ANOVA, $F_{(5,84)} = 50.5$ six vs. end (week twel n.s. $p = .054$, 100 m the performance of tests (Fig. 2 C-D). reaction influen these tests.

The open motor locom E, RD2 trea explorator genotype $p < .00$ Fig. 2 E

3.3. P reveal

agg wit ca A ar p g r s

litt The incr mat



f Aβ particles of RD2-treated homozygous (hom) TBA2.1 mice in different brain region
e Aβ deposits (staining with 6E10 antibody with subsequent quantification of Aβ particle
f 100 mg/kg/day RD2-treated hom TBA2.1 mice in comparison to placebo treated mi
egions are given on the right. Data is represented as mean ± SEM, two-way ANOVA w

as detectable, irrespective of the analysed cell type or brain derpinning that RD2 treatment did not cause any additional n inflammatory cells (Table 1).

tionally, an $A\beta_{(x\text{-}42)}$ ELISA was conducted with brain homo- s in order to measure the total amount of $A\beta_{(Q3\text{–}42)}$ and $_{(3\text{–}42)}$. This resulted in a significant decrease in $A\beta_{(x\text{-}42)}$ in the Tris- ble fraction of RD2-treated homozygous TBA2.1 mice compared to cebo-treated mice (two-way ANOVA, $F_{(2,78)} = 3.57$ $p = .033$, Fisher SD post hoc analysis placebo vs. 20 mg/kg/day $p \leq .001$, placebo vs. 100 mg/kg/day n.s. $p < .001$, 20 mg/kg/day vs. 100 mg/kg/day n.s. $p = .98$, Fig. 4 A). While no difference in the amount of Aβ species in the DEA-fraction was detected, a significant increase of insoluble Aβ species within the FA-fraction of both RD2 treatment groups was shown in comparison to placebo-treated mice (two-way ANOVA, $F_{(2,78)} = 3.57$ $p = .033$, Fisher LSD post hoc analysis placebo vs. 20 mg/kg/day $p \leq .001$, placebo vs. 100 mg/kg/day n.s. $p = .002$, 20 mg/kg/day vs. 100 mg/kg/day $p = .02$, Fig. 4 A).

Furthermore, a $pEA\beta_{(3\text{–}42)}$ specific ELISA was performed to investigate, whether RD2 treatment had any influence on $pEA\beta_{(3\text{–}42)}$ concentrations in different brain homogenate fractions. The

biochemical an DEA-soluble f placebo- and an significa treated mi post hoc placebo day p placeb day p the F One pEA wel hu Aβ

tw la w

Table 1
Immunohistochemical investigations of RD2 treatment on neurodegeneration, and ne neurodegeneration (staining with NeuN antibody with subsequent quantification of compared to placebo-treated homozygous TBA2.1 mice. Immunostaining using antibo microglia, respectively, to analyse a possible change of inflammatory processes after t region CA1, inferior colliculus; CD11b inferior colliculus, brain stem). No significa cortex. IR: immunoreactivity. Data is represented as mean ± SEM, two-way ANOV

	Staining	Brain region	Placebo	
Homozygous TBA2.1	NeuN (IR [%])	Striatum	16.2 ± 0.8	
		MC	38.3 ± 2.8	
		CA1	10.6 ± 0.6	
	GFAP (IR [%])	MC	22.4 ± 1.5	
		CA1	36.2 ± 1.5	
	CD11b (IR [%])	IC	0.86 ± 0.2	
		Brain stem	0.2 ± 0.03	
Non-transgenic littermates	NeuN (IR [%])	Striatum	39.6 ± 2.0	3
		MC	42.0 ± 0.4	4
		CA1	26.9 ± 5.1	2
	GFAP (IR [%])	MC	8.8 ± 1.6	6.
		CA1	6.3 ± 0.7	3.1
			0.44 ± 0.3	0.7
		stem	0.34 ± 0.2	0.1

reatment on $A\beta_{(x\text{-}42)}$ and $pEA\beta_{(3–42)}$ levels in brains of nice. Levels of $A\beta_{(x\text{-}42)}$ and $pEA\beta_{(3–42)}$ were analysed in mine (DEA)-, and formic acid (FA)-fractions by ELISA of reated homozygous TBA2.1 mice. Analysis of $A\beta_{(x\text{-}42)}$ re- ant decrease of soluble $A\beta_{(x\text{-}42)}$ in the Tris-fraction of 20 and reated homozygous TBA2.1 mice, compared to their placebo es. Furthermore, a significant increase of insoluble $A\beta_{(x\text{-}42)}$ in of 20 and 100 mg/kg/day homozygous TBA2.1 mice was de- nalysis of $pEA\beta_{(3–42)}$ revealed a significant increase in the DEA- mg/kg/day RD2-treated mice compared to placebo- and 100 mg/ 2-treated mice. Concentrations are given as pg $pEA\beta_{(3–42)}$/g brain. presented as mean ± SEM, two-way ANOVA with Fisher post hoc $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$.

elated significantly with a decrease of soluble $A\beta_{(x\text{-}42)}$ species = .01), as well as with increased insoluble $A\beta_{(x\text{-}42)}$ (p = .03) able 2). No significant correlations were observed between the $pEA\beta_{(3–42)}$ concentrations in either brain fraction, or $A\beta_{(x\text{-}42)}$ in the DEA-faction and the SHIRPA scores of the mice (Table 2).

4. Discussion

Convincing evidence suggests a crucial role for Aβ oligomers for the development and progression of AD (Ferreira and Klein 2011; Ferreira et al. 2015; Haass and Selkoe 2007a; Salahuddin et al. 2016; Selkoe and Hardy 2016; Wang et al. 2016). Thus, compounds that eliminate Aβ oligomers are thought to be an auspicious treatment option (Rosenblum 2014). RD2, a compound solely consisting of D-enantiomeric amino acid residues, was developed for the specific and direct elimination of toxic Aβ oligomers. The Aβ oligomer elimination efficacy of RD2 was demonstrated in vitro and in vivo, as well as the efficacy to improve cognitive deficits in two AD mouse models (APP/PS1 and APP_{SL}), without changing the typical AD-associated pathology (Aβ plaque load, inflammation) (Kutzsche et al. 2017; Schemmert et al. 2018; van Groen et al. 2017). The purpose of the current study was to add an AD mouse model to the in vivo efficacy portfolio of RD2, which expresses a highly neurotoxic Aβ species. Homozygous TBA2.1 mice are expressing $A\beta_{(Q3–42)}$, producing the extremely aggregation-prone Aβ species $pEA\beta_{(3–42)}$, thereby developing small intracellular Aβ aggregates and a

Table 2
Correlation between SHIRPA Scores and $A\beta_{(x\text{-}42)}$ or $pEA\beta_{(3–42)}$ levels.

	$A\beta_{(x\text{-}42)}$		
	Tris	DEA	FA
SHIRPA-Score	$r = 0.51^{**}$	$r = -0.17$	$r = -0.46$

r = Pearson co

*

ated, because so far it is not known, whether
elop cognitive deficits. In any case, cognitive be-
nts for TBA2.1 mice should be selected and per-
reatest possible caution, because most standard cog-
Morris water maze, any other maze, novel object
pend on functional movement capabilities of the ani-
2.1 mouse model, however, develops a severe motor-
ative phenotype making it hard to dissect the outcome of
periments from changes in the motor-neurodegenerative

estigate the causal reason for the decelerated progression of
otype of homozygous TBA2.1 mice due to RD2 treatment, we
ed additional immunohistochemical and biochemical analyses.
nohistochemical investigations of neurodegeneration, Aβ pa-
gy, and gliosis in the brains resulted in no significant difference
ween all tested groups in all analysed regions. By trend, a reduction
Aβ particles within the striatum of RD2-treated mice (100 mg/kg/
day) was measureable. Thus, a reduction of Aβ particles and accom-
panied neuroinflammation in brain seemed not to be the main mode of
action. This is in accordance with previous results in other AD mouse
models after treatment with RD2 (Kutzsche et al. 2017; van Groen et al.
2017). However, a significant effect on neuronal loss would have been
expected, as RD2 treatment led to a significant deceleration of the
motor-neurodegenerative phenotype of homozygous TBA2.1 mice. Pa-
thologically, we could not demonstrate a significant effect on neuro-
degeneration, i.e. neuron count, in this study. This is a limitation of the
study as it is still unclear on which level RD2 rescues neuronal function.

By using an $A\beta_{(x\text{-}42)}$ specific ELISA, we were able to give further
hints to the potential mode of action of RD2. We suggest that RD2 binds
to monomeric $A\beta_{(1–42)}$ (here: $A\beta_{(Q3–42)}$) and $pEA\beta_{(3–42)}$, thus shifting
the equilibrium from toxic Aβ oligomers away either to non-toxic Aβ
monomers or other non-toxic Aβ RD2 co-assemblies, which at least
under non-physiological in vitro conditions are high molecular weight,
non-toxic, and non-amyloidogenic co-precipitates (Funke et al. 2010).
Those co-precipitates, if formed in vivo too, can be hypothesized to be
found in the formic acid-fraction of the brain homogenates. Due to this,
there is a significant increase of insoluble $A\beta_{(Q3–42)}$ or $pEA\beta_{(3–42)}$, which
in turn should not be equated with an increase in fibrillary Aβ. Ac-
cording to this hypothesis, we conclude successful in vivo target en-
gagement underlined by the results of the $A\beta_{(x\text{-}42)}$ specific ELISA. We
were able to demonstrate a significant reduction of soluble Aβ species
in the Tris-fraction, which also contains soluble Aβ oligomers, at both
administered RD2 doses and an increase in the insoluble formic acid-
fraction. We assume that the deceleration of the motor-neurodegen-
erative phenotype is rather based on a reduction of oligomeric Aβ than
a change of monomeric $A\beta_{(Q3–42)}$ or $pEA\beta_{(3–42)}$. However, since we
cannot distinguish between monomeric and oligomeric Aβ in the Tris
fraction, these results could also be interpreted as reduction of Aβ
monomers by tr
ndication to assume that
the
ated mice represents
brains of TBA2.1

international, national, and/or institutional guide- and use of animals were followed. All procedures udies involving animals were in accordance with the ds of the institution or practice at which the studies were l animal experiments were performed in accordance with Law on the protection of animals (TierSchG §§ 7–9) and oved by a local ethics committee (LANUV, North-Rhine- a, Germany, reference number: Az: 84-02.04.2014.362 and 2.04.2011.A359).

or contributions

A.W., S.S., D.W., and J.K. planed the overall study. S.S. performed nd analysed the treatment study, ELISA experiments, and immunohistochemistry with help of E.S. and D.H. T.Z. conducted the SPR measurements and ThT assays. S.S., A.W., and D.W. wrote the initial manuscript with scientific advice of J.K., C.Z., N.J.S. and K-J.L.. All authors contributed to writing and reviewed the manuscript.

Acknowledgements

We thank Probiodrug for providing us the TBA2.1 mice. Additionally, we thank the Forschungszentrum Jülich animal facility for their excellent care.

References

Alexandru, A., et al., 2011. Selective hippocampal neurodegeneration in transgenic mice expressing small amounts of truncated Abeta is induced by pyroglutamate-Abeta formation. J. Neurosci. 31, 12790–12801.

Bayer, T.A., Wirths, O., 2014. Focusing the amyloid cascade hypothesis on N-truncated Abeta peptides as drug targets against Alzheimer's disease. Acta Neuropathol. 127, 787–801.

Brener, O., et al., 2015. QIAD assay for quantitating a compound's efficacy in elimination of toxic Abeta oligomers. Sci. Rep. 5, 13222.

Dammers, C., et al., 2015. Purification and characterization of recombinant N-terminally pyroglutamate-modified amyloid-beta variants and structural analysis by solution NMR spectroscopy. PLoS One 10, e0139710.

Dammers, C., et al., 2017a. Pyroglutamate-modified amyloid-beta(3-42) shows alpha-helical intermediates before amyloid formation. Biophys. J. 112, 1621–1633.

Dammers, C., et al., 2017b. Pyroglutamate-modified Abeta(3-42) affects aggregation kinetics of Abeta(1-42) by accelerating primary and secondary pathways. Chem. Sci. 8, 4996–5004.

Dunkelmann, T., et al., 2018a. Comprehensive characterization of the pyroglutamate amyloid-beta induced motor neurodegenerative phenotype of TBA2.1 mice. J. Alzheimers Dis. 63, 115–130.