Erfolgsbericht:

Projekt: **Chemoprophylaxe und Chemotherapie bei Prion-Infektionen**

[R:PRIONBMS]

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Wie schon in unserem Projektantrag aus dem Jahre 1994 festgestellt, so gilt auch noch heute, daß keine effektive Therapie der Prionerkrankungen vorhanden ist. Uns ist es erstmals gelungen zu zeigen, daß bei der Induktion des Nerventodes bei Prionerkrankungen ein erhöhter Einstrom von Ca^{2+} in die Zellen eine entscheidende Rolle spielt. Deshalb war es auch folgerichtig, daß wir als erste Gruppe einen Weg für eine Therapie aufzeigen konnten. Basierend auf diesen Vorarbeiten und entsprechend den förderpolitischen Zielen, wurde von uns der o.g. Antrag gestellt, mit der Zielsetzung eine effektive Chemoprophylaxe bei Prionerkrankungen auszuarbeiten.

Zwei Substanzen, die klinisch einsetzbar sein könnten, wurden von uns bearbeitet: • Memantin (ein NMDA-Antagonist) und • Flupirtin (ein neuartiges neuroprotektives Medikament).

Bezüglich Memantin war eine vorklinische Tieruntersuchung aufgrund ungünstiger pharmakokinetischer Daten nicht möglich.

Flupirtin wurde in seiner Wirkung molekular aufgeklärt. Es wurde ein neuer Angriffspunkt gefunden [Modulation der *Bcl-2*-Expression], der auch von anderen Arbeitsgruppen im Tierversuch bestätigt wurde.

Hauptsächlich aufgrund unserer Untersuchungen wurde eine klinische Phase 3-Studie bei Creutzfeldt-Jakob-Erkrankungen mit Flupirtin begonnen. Dies ist weltweit die erste klinische Studie bei diesem Krankheitsbild.

Ingesamt gesehen erachten wir die Ergebnisse, die bisher im Rahmen dieser Untersuchungen erarbeitet wurden, als international herausragend; diese Ergebnisse sind in international hervorragenden Journalen publiziert worden.

Ein weiterer Antrag auf finanzielle Förderung der von uns betriebenen Arbeiten mit dem Projekttitel "Entwicklung neuroprotektiver Substanzen zur Behandlung von Prion-Erkrankungen: Zweite Generation" wurde nicht genehmigt.

Eine ausführliche Beschreibung des wissenschaftlichen Erfolgs dieses Projekts ist im Schlußbericht zusammengefaßt, der dem Projektträger vorliegt. Eine

Zusammenfassung ist - entsprechend den Vorgaben zu dem damaligen Aufruf - in Englisch gehalten.

1.1. Summary: aims, methods, results, conclusion

- Goal 1. Elucidation of the interaction of the prion protein with neurons and its consequence
- Goal 2. Screening for and subsequent development of chemotherapeutics potentially applicable in the treatment of prion diseases in humans

• ***Methods:*** Cellular- [neurons *in vitro*], subcellular techniques.

• ***Results:*** **Goal 1:** One major RNA-binding brain protein, the ß-galactoside-specific CBP35, binds to PrP mRNA; to this complex PrP can associate. Small amounts of CBP35 are also present at the surface of cells, which apparently interact with extracellular PrP^{Sc}. Both PrP^{Sc} and PrP106-126 induce apoptosis in neuronal cells by sensitization of the NMDA receptor. **Goal 2: Memantine,** an NMDA receptor antagonist, previously found to protect neurons *in vitro* against PrP^{Sc}-mediated apoptosis, is in the progress to be tested in animal experiments [scrapie-infected hamsters]. As a pragmatic choice the animals will be treated with **memantine**, applied in food pellets after a tolerance study. **Flupirtine,** a drug used in clinics as a centrally acting, non-opiate analgesic agent, was found to display potent cytoprotective activity on neurons *in vitro*, treated with the prion protein fragment PrP106-126. It was demonstrated that flupirtine prevents the apoptotic process by two mechanisms in *in vitro* cultures: by normalization of the GSH level and by induction of *Bcl-2*.

• ***Conclusion:*** **Goal 1:** PrP^{Sc} may bind to neurons via a lectin interaction. **Goal 2:** The animal study with **memantine** is under way. **Flupirtine, is the candidate for clinical trials in humans** due to (i) our *in vitro* data on the neuroprotective activity against PrP106-126, and (ii) the already existing *in vivo* results in further neuroprotective models [e. g., focal cerebral ischemia (mouse), global cerebral ischemia (rat) and retinal ischemia (rat and rabbit)] as well as (iii) the favourable pharmacokinetic properties**; one trial is planned to be started soon**. Until now, no clinical studies on prion diseases in humans by other groups have been conducted.

• ***Future strategies:*** Special emphasis is laid on **new approaches in screening for chemotherapeutics potentially applicable in the treatment of prion diseases in humans.** The ***facilities*** to perform the planned experiments [e.g. P2- and P3-laboratories] are available.

1.2. Publications during funding period

1. S. Perovic, G. Pergande, H. Ushijima, M. Kelve, J. Forrest and W.E.G. Müller: Flupirtine Partially Prevents Neuronal Injury Induced by Prion Protein Fragment and Lead Acetate. Neurodegeneration 4, 369-374 (1995).
2. S. Perovic, P. Pialoglou, F.J. Romero, G. Pergande and W.E.G. Müller: Flupirtine Increases the Levels of Glutathione and *Bcl-2* in NT2 (human Ntera/d1) Neurons: Mode of Action of the Drug-mediated Anti-apoptotic Effect. Europ. J. Pharmacol., 317, 157-164 (1996).
3. W.E.G. Müller, F.J. Romero, S. Perovic, G. Pergande and P. Pialoglou: Protection of Flupirtine on ß-Amyloid-induced Apoptosis in Neuronal Cells in vitro: Prevention of Amyloid-induced Glutathione Depletion. J. Neurochem., 68, 2371-2377 (1997).
4. W.E.G. Müller, G. Pergande, C. Schleger, H. Ushijima and S. Perovic: Neurotoxicity in Rat Cortical Cells Caused by N-Methyl-D-Aspartate (NMDA) and Gp120 of HIV-1: Induction and Pharmacological Intervention. Progr. Molec. Subcell. Biol. 16, 44-71 (1996).
5. H.C. Schröder, U. Scheffer, J. Leuck, T. Sklaviadis, S. Perovic, A.-P. Sève, J.M. Leitão and W.E.G. Müller: Glycoprotein Lectin Interactions of Prion Protein. Possible Roles in Pathogenesis of the Disease Process Caused by Scrapie Prion Protein. In: (E. van Driessche, P. Rougé, S. Beeckmans and T.C. Bøg-Hansen; eds.) Lectins: Biology, Biochemistry, Clinical Biochemistry; vol. 11; Textop, Helerup Ltd. (Denmark), pp. 293-306 (1996).
6. U. Scheffer, T. Okamoto, J.M.S. Forrest, P.G. Rytik, W.E.G. Müller and H.C. Schröder: Interaction of 68-kDa TAR RNA-binding Protein and Other Cellular Proteins with Prion Protein-RNA Stem-Loop. J. NeuroVirology 1, 391-398 (1995).
7. U. Scheffer: Prionen. Medizinische Klinik. 90, 653-657 (1995).
8. W.E.G. Müller, U. Scheffer, S. Perovic, J. Forrest and H.C. Schröder: Interaction of Prion Protein mRNA with CBP35 and other Cellular Proteins: Possible Implications for Prion Replication and Age-dependent Changes. Arch. Gerontol. Geriatrics; in press.
9. W.E.G. Müller, F.J. Romero, S. Perovic, G. Pergande and P. Pialoglou: Protective Effect of the Drug Flupirtine on ß-Amyloid-Induced Apoptosis in Primary Neuronal Cells *in vitro*. J. Brain Res. 37, 575-577 (1996).
10. H.C. Schröder, H. Ushijima, C. Theis, A.-P. Sève, J. Hubert and W.E.G. Müller: Expression of Nuclear Lectin Carbohydrate-binding Protein 35 in Human Immunodeficiency Virus Type 1-infected Molt-3 Cells. J. Acqu. Imm. Def. Syndr. Human Retrovir. 9, 340-348 (1995).

11. A. Kuusksalu, E. Truve, A. Aaspollu, M. Kelve, U. Scheffer, W.E.G. Müller and H.C. Schröder: Impairment of Intracellular Antiviral Defense with Age: Age-dependent Changes in Expression of Interferon-induced and Double-stranded RNA-activated 2-5A Synthetase in Rat. Mech. Ageing Develop. 78, 103-115 (1995).
12. S. Perovic, H.C. Schröder, G. Pergande, H. Ushijima and W.E.G. Müller: Downregulation of *Bcl-2* and Glutathione Level in Neuronal Cells *in vitro* Treated with the Prion Protein Fragment (PrP106-126): Prevention by Flupirtine. Neurodegeneration; submitted.
13. H. Ushijima, O. Nishio, R. Klöcking, S. Perovic and W.E.G. Müller: Exposure to gp120 of HIV-1 Induces an Increased Release of Arachidonic Acid in Rat Primary Neuronal Cell Culture Followed by NMDA Receptor Mediated Neurotoxicity. Europ. J. Neurosci. 7, 1353-1359 (1995).
14. W.E.G. Müller, J.M. Dobmeyer•, Th.S. Dobmeyer•, G. Pergande S. Perovic, J. Leuck and R. Rossol: Flupirtine Protects Both Neuronal Cells and Lymphocytes to Undergo Induced Apoptotic Death *in vitro*: Implications for Treatment of AIDS patients. Death & Differentiation 4, 51-58 (1997).
15. S. Perovic, M. Böhm, H.C. Schröder, G. Pergande and W.E.G. Müller: Pharmacological Intervention in Age-associated Disorders: Alzheimer's- and Prion Diseases. Flupirtine Reduces Apoptosis of Neurons *in vitro.* Mech. Ageing Develop.; submitted.
16. W.E.G. Müller, S. Perovic, J. Leuck, G. Pergande und H.C. Schröder: Neuronaler Zelltod: Schutz durch Flupirtin. Forschungsmagazin [der Joh. Gutenberg Universität; Mainz]; Sonderheft BioRegio 12, 24-37 (1996).

Description of the project

1.3. Specific aims

The general aim of this project is the outline and the experimental elucidation of strategies to interfere with prion infections, especially in humans. *In this regard our project is the only one in this BMBF "Verbund".*

A therapeutic intervention against prion diseases does not exist. Thus, an urgent need exists for compounds displaying therapeutical potential, especially at present.

To achieve this goal the following strategies have been formulated and experimentally followed:

a. Elucidation of the interaction of the prion protein with neurons and its consequence

b. Screening for and subsequent development of chemotherapeutics potentially applicable in the treatment of prion diseases in humans

This program is an integrated approach, combining data resulting from basic research activities with applicable oriented efforts. Special emphasis is given to the development of (a) chemotherapeutic agent(s) potentially suitable for the treatment of prion diseases. *Hence, this individual project fulfills the demands of the BMBF program for an applied research activity in the field of infectious diseases, here of "prion diseases".*

1.4. Introduction and *own work* prior the establishment of the "Verbund"

(The references cited from our group are marked in italics)

The proteinaceous infectious particles, termed prions (PrP), have been implicated in the pathogenesis of a number of both animal and human neurodegenerative disorders. Two isoforms of PrP exist, the normal host protein, designated PrP^{C}, and the modified PrP, PrP^{Sc}, which is present in the infectious scrapie particles. In humans both PrP^{Sc} and PrP^{C} are encoded in a single exon of a single-copy gene. The function of PrP^{C} is not known; it is found predominantly on the surface of neurons, attached by a glycoinositol phospholipid anchor, while PrP^{Sc} has been described as accumulating intracellularly in cytoplasmic vesicles.

1.4.1. Elucidation of the interaction of the prion protein with neurons and its consequence

1.4.1.1. Interaction of prion protein with intracellular proteins

In the sequence of the PrP mRNA three sets of stem-loop structures are found which have the pentanucleotide CUGGG in the loop and the Ura- and Ade bulge in the stem; these features are also characteristic for the TAR sequence of HIV-1. The TAR element of HIV-1 mRNA is the target sequence for Tat. The TAR stem-loop is also recognized by certain host cell proteins (Gatignol et al., 1989; *Schröder et al., 1990; Müller et al., 1990*).

Evidence has been presented that certain nuclear lectins, carbohydrate-binding proteins (CBPs), including the ß-galactoside-specific CBP35 and the glucose-specific CBP67, are associated with or are constituents of nuclear ribonucleoprotein (RNP) complexes (*Schröder et al., 1992*; Wang et al., 1992). Nuclear lectins have been found to associate with RNA directly or indirectly via an RNA-binding protein: protein interaction (*Lauc et al., 1993*).

During the course of our studies, we described that PrP mRNA stem-loops associate with a set of proteins. CBP35, which was among the PrP mRNA-binding