CNTF reduces cognitive impairment in a mouse model of Alzheimer's disease

J.K. Utvik[1], I. Youssef[3], B. Kriem[3], P. Garcia[3], A. Oudin[1], T. Oster[3], R. Bjerkvig[1&2], T. Pillot[3] and S.P. Niclou[1]
[1] CRP-Santé, Luxembourg [2] University of Bergen, Norway [3] Institut National Polytechnique de Lorraine, Nancy, France (Contact: simone.niclou@crp-sante.lu)

Introduction

Alzheimer's disease (AD) is a devastating progressive neurodegenerative disease and its incidence is rising in Western countries due to increased life expectancy. Recent data supports the idea that soluble oligomers of amyloid-β peptide (sAβ) are highly neurotoxic and represent a key factor in the early stages of neurodegeneration leading to AD (Walsh and Selkoe, 2007). We hypothesise that *in vivo* expression of a neuroprotevtive agent **prevents neurodegeneration** and subsequent **cognitive impairment** in AD. The aim of this project is to evaluate the efficacy of ciliary neurotrophic factor (CNTF) in sAβ injected mice after stereotaxic implantation of **alginate-encapsulated recombinant cells**, providing local and continuous delivery of CNTF to the diseased brain.

The limited passage of drugs through the blood brain barrier and the short half-life of locally injected therapeutic molecules are major hurdles in the treatment of neurodegenerative diseases. **Cell-based delivery systems**, such as **cell micro-encapsulation** devices, provide long term delivery of the biologically active compound *in situ* and are a promising strategy for therapeutic applications in the brain (Emerich and Salzberg, 2001; Niclou and Bjerkvig, 2008; Utvik and Niclou, 2008). The encapsulation of the cells in naturally occurring hydrogels (e.g. alginate-based gels) prevents the immune system from destroying the transplanted cells, thus allowing the use of non-autologous cells for **recombinant cell therapy** (Bjerkvig et al., 2003; de Vos et al., 2006). The present project will open up new avenues for the use of micro-encapsulation technology as a new strategy in the treatment of Alzheimer's disease.

The **specific aims** of the project are: **1 –** To optimise the implantation of CNTF-secreting cells encapsulated in alginate beads in the brain of a mouse model of AD - and to monitor peptide production and diffusion over time. **2 –** To characterise the effects of CNTF production in the brain on both cellular and tissular injuries associated with the AD brain, as well as on the cognitive performances of treated mice.

CNTF belongs to the IL-6 family of cytokines and is known for its neuroprotective effects, being a survival factor for sympathetic, sensory, hippocampal and motors neurons *in vitro* as well as *in vivo*. Exogenous CNTF protects mature neurons from degeneration arising from multiple etiologies including Huntington's disease, amyotrophic lateral sclerosis or retinal degeneration and its efficacy is currently tested in several clinical trials (Bongioanni et al., 2004; Emerich and Thanos, 2006; Silani et al., 2001). Despite the promise of CNTF in treating neurodegenerative disorders, it has not been evaluated for pre-clinical use in the context of AD pathology. The data presented are thus novel and promising, and fully justify the evaluation of CNTF released from microcapsules as a novel therapy for AD.

Material and Methods

Encapsulation of CNTF-expressing cells: Cell lines stably expressing CNTF (C2C12 myoblasts and BHK baby hamster kidney cells; gift from P. Aebischer) were genetically engineered by lentiviral

vector transduction to express a fluorescent marker protein: green fluorescent protein (GFP) or Discosoma red fluorescent protein (DsRed). This allows the direct visualization of the capsules *in vitro* and *in vivo* and to follow cell survival over time. The producer cells (C_2C_{12} or BHK) were harvested and mixed into a 2% sodium alginate (ultrapure, low viscosity, high guluronic acid content; PRONOVA (TM) UP LVG) saline solution (0.9% NaCl, 10mM MOPS, pH 7.4) to give a concentration of 50×10^6 cells/ml alginate solution. Beads were generated with an electrostatic bead generator. Sharpened nozzles with diameter of 0.17 mm were used (Nisco Engineering AG, Switzerland). The gelling bath was composed of 0.1M $CaCl_2$ in 0.9% NaCl with 10mM MOPS, pH 7.4. After encapsulation the cell line released CNTF from the micro-capsules, verified by Western blot analysis and ELISA (data not shown).

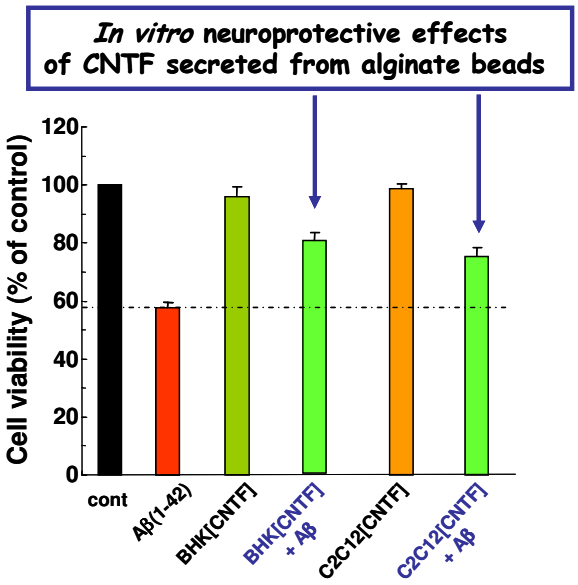

Figure 1 – *In vitro* neuroprotective effects of recombinant CNTF produced from alginate encapsulated cells. Mouse hippocampal neurons were incubated for 2h with capsule conditioned medium, before addition of 1µM sAβ oligomers. Cell viability was monitored after 24h with the MTT assay.

Animal model: Mice are stereotaxically injected with 50 pmol soluble Aβ oligomers in the ventricule as previously described (Youssef et al. 2007).

Neuroprotective effect of CNTF *in vitro*: Neuroprotection of sAß oligomer-induced neuronal cell death was performed as previously described (Kriem et al., 2005).

Analysis of C2C12 encapsulated cells in vivo: Micro-capsules with C2C12-CNTF cells were injected unilaterally in the subhippocampal region or into the lateral ventricle of wildtype mice (1 or 3 capsules per mouse). No surgery-related problems and no behavioural defects were observed in the mice (data not shown). CNTF secretion and its diffusion into the CSF and the brain parenchyme are investigated at different time points (3 weeks, 5 weeks and 3 months post-implantation) by ELISA and immunohistochemistry. Cell survival in the beads will be evaluated on histological sections. At the same time blood is retrieved to test for production of antisera to human CNTF in mouse blood. These studies are ongoing.

Behavioral tests: *MORRIS water Maze* - The Morris water maze is a behavioural procedure designed to test spatial memory. Memory-acquisition trials (training) are performed four times daily on day 7–11 post surgery to reach a steady state of escape latency. Memory-retention tests (probe trials) are performed 3 days after the last training session. *The Y maze* - Immediate spatial working memory performance are assessed by recording spontaneous alternation behaviour in a Y-maze. Alternation is defined as successive entries into the three arms on overlapping triplet sets. The percentage alternation will be calculated as the ratio of actual (total alternations) to possible alternations (defined as the number of arm entries minus two), multiplied by 100.

Results and Discussion

Neuroprotective effect of CNTF *in vitro*: We show that the conditioned medium from encapsulated CNTF cells prevents soluble Aβ oligomer-induced neuronal death *in vitro* (**Figure 1**).

Effect of CNTF on cognitive performance of sAβ challenged mice: We then assessed the effect of CNTF cell capsules on the cognitive performance in the sAb injected AD mouse model (for details on the model see (Youssef et al., 2007). In this large scale *in vivo* experiment, mice received first hippocampal implants of C2C12-CNTF micro-capsules followed three weeks later by a single injection of soluble Aβ oligomers. Working memory and longterm memory were subsequently evaluated with appropriate behavioural tests (Y maze and Morris water maze). The experimental setup used in these experiments is described in **Figure 2**.

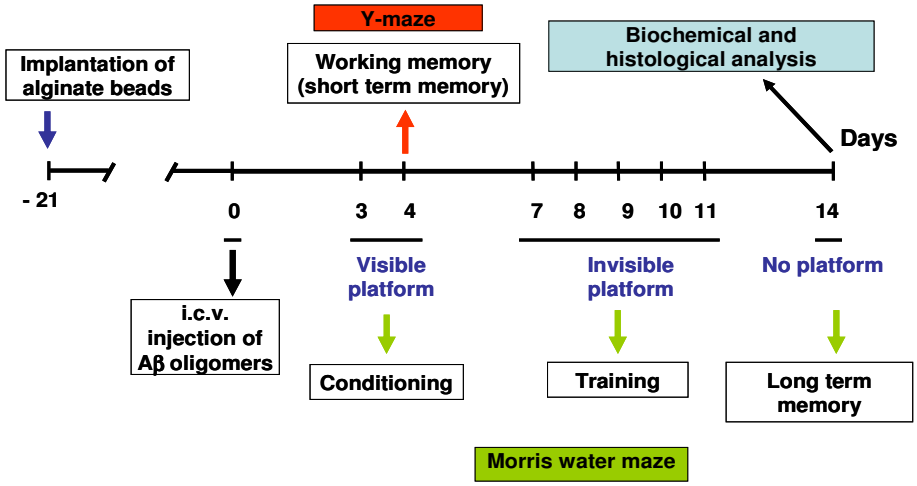

Figure 2 – Experimental procedure used for the evaluation of neuroprotective effects of CNTF secreted from implanted encapsulated cells. One bead containing encapsulated C2C12-CNTF or C2C12 (control) cells were stereotaxically implanted in the subhippocampal region of mice brain (15 animals per group) 3 weeks before i.c.v. injection of 50 pmol soluble Aβ oligomers. Working memory and long-term memory were assessed using the Y maze and the Morris water maze tests, respectively as previously described (Youssef et al., 2007).

Our results indicate that CNTF secreted from implanted recombinant cells results in strong protection against soluble Aβ oligomer-induced impairment of spatial memory monitored using the Y maze test (**Figure 3**). Moreover, secreted CNTF also protects implanted animals from sAβ peptide-induced impairment of learning and long term memory in the Morris water maze test (**Figure 3**). Preliminary experiments were also performed with intraventricular capsule implants which also lead to an improvement of cognitive performance in both the Y maze and Morris water maze (data not shown).

The *in vivo* experiments will be corroborated by addressing the cognitive alterations at the cellular, molecular and biochemical level. One explanation for the fast behavioral deterioration observed in mice injected with sAβ is an alteration or reduction of synaptic contacts. To test this hypothesis the expression of several synaptic marker proteins is investigated after sAß application.

CONCLUSIONS

We have shown that **encapsulated CNTF secreting cells protect mice from soluble Aβ oligomer-induced behavioral and memory impairment** (*manuscript in preparation*). To our knowledge this is the first time that a beneficial effect of CNTF on AD-related pathology is reported. It is worthy to note that the implantation of alginate beads in the lateral ventricle of mice results in a

similar restoration of cognitive performance than the hippocampal implants. This should allow us to optimize the site of bead implantation in order to obtain maximal effects. Importantly, we have made a **first proof of concept** that CNTF secreted from **alginate encapsulated recombinant C2C12 cells** protects from soluble Aβ oligomer-induced cognitive impairment in a mouse model of AD.

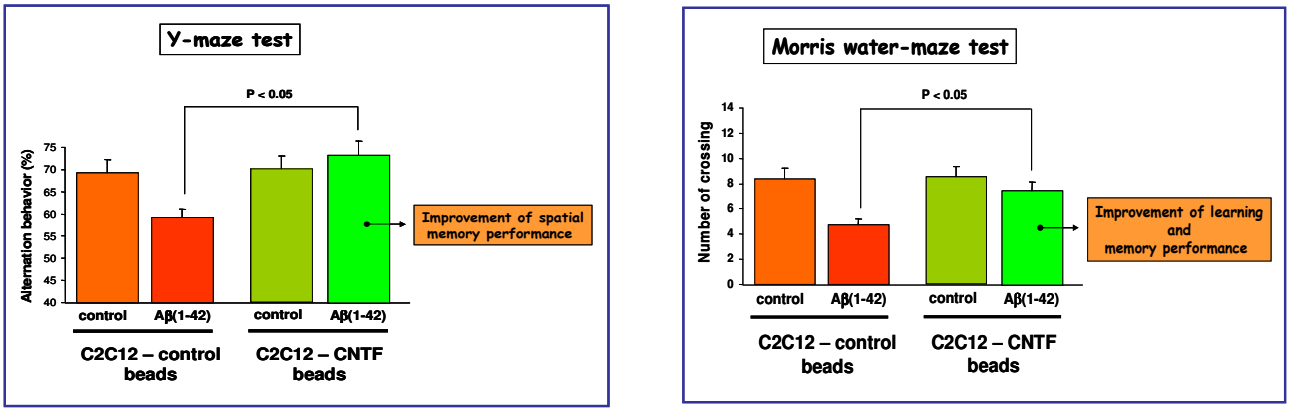

Figure 3 – *In vivo* neuroprotective effects of recombinant CNTF produced from implanted alginate beads. One bead containing encapsulated C2C12-CNTF or C2C12 (control) cells have been stereotaxically implanted in the subhippocampal region of mice brain (15 animals per group) 3 weeks before i.c.v. injection of 50 pmol soluble Aβ oligomers. Working memory and long-term memory have been assessed using the Y maze and the Morris water maze tests, respectively.

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