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# Therapeutic potential of non-neuronal cells in Amyotrophic Lateral Sclerosis (ALS)

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## Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the death of motoneurons in the motor cortex, brain stem and spinal cord. The G93A transgenic mouse model expresses a mutant form of human superoxide dismutase (SOD1) and develops motoneuron disease similar to ALS (*Rosen, Siddique, 1993*). It has previously been shown that surrounding non-neuronal cells (glia) have an influence on the survival of motoneurons (*Clement et al., 2003; Nagai et al., 2007*). We are therefore currently studying the interaction of neuronal and non-neuronal cells in vitro using a co-culture system.
In this system, we have addressed the potential neuroprotective capacities of Glucagon-like peptide-1(GLP-1). GLP-1 has initially been studied as a treatment for type II diabetes based on its major function as insulin secretagogue (*John et al,2008*). Neuroprotective capacities of GLP-1 are supposed to arise from a direct effect on cell membrane as well as an effect on local synaptic circuits (*Wan et al.,2007*).

## Methods

### Primary Culture

The isolation and in vitro cultivation of embryonic primary motoneurons from individual mouse embryos (gestational age:E14/15 ) were performed by a p75NTR-antibody-based immunopanning technique. Highly enriched motoneuron preparations were seeded on glass coverslips either pre-incubated with poly-L-Lysin (diluted 1:1000; Sigma) for co-culture or pre-incubated with laminin (2,5µg/ml; Invitrogen) for monoculture.

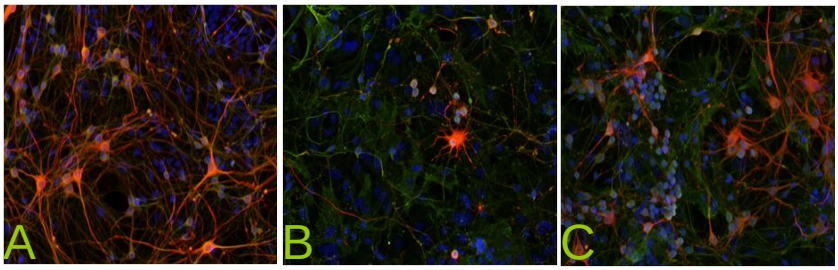
### Toxicity Experiments

Motoneuron-rich cell fractions (MN) derived from either wildtype or mutant G93A transgenic ALS mice (WT MN/ G93A MN) were seeded on laminin or on glial feeder layers of wildtype or transgenic neonatal mouse astrocytes (WT A/ G93A A) at an average density of 2.0x $10^4$ cells /$cm^2$. After 7 days in vitro (DIV 7), cell cultures were incubated for 24h with 300µM KA. Furthermore, 300µM KA was applied, together with 100nM GLP-1, for 24 h, to test for its possible neuroprotective effect.

### Immunocytochemistry

Cells were fixed with 4% para-formaldehyde (PFA). Glial feeder layers were stained with antibodies against glial fibrillary acidic protein (GFAP) (1:50; Sigma, Germany) to identify astrocytes. Motoneurons were stained with an antibody against SMI32 (1:100; Covance, USA) which is specific for motoneurons derived from embryonic spinal cord. The nuclei of all cultured cells were stained with 4',6-diamidino-2-phenylindole (DAPI; Sigma). Cell counts were done in 5 visual fields of each coverslip in a total of 5-7 different preparations for each condition. Counting was performed using an ocular counting grid to quantify all cells on a horizontal stripe crossing the complete well, using a fluorescence microscope (BX-70).

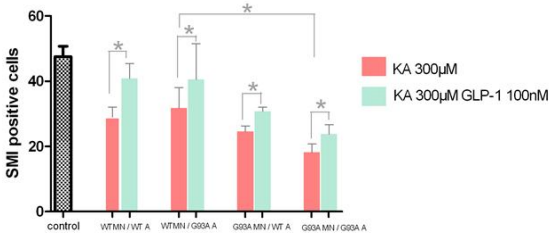
## Results




**untreated** **KA** **KA + GLP-1**

**Figure 1**

Reduction of SMI32 positive motoneurons following KA exposure. Increase in cell survival due to GLP-1 co-incubation. Motoneurons were stained using antibodies against SMI-32 (red). Astrocytes were immunopositive for GFAP (green). Stained nuclei of cultured cells appear in blue (DAPI).




* p< 0.05

**Figure 2**

GLP-1 protected motoneurons from toxicity induced by KA.

G93A motoneurons in co-culture with G93A astrocytes were significantly more sensitive to KA-toxicity than wildtype motoneurons in co-culture with either wildtype or transgenic astrocytes.

## Conclusion

Under KA exposure, there was a a significantly increased sensibility of the G93A transgenic motoneurons compared to the wildtype ones ($p<0.05$). When comparing the co-culture of G93A MN/WT A and G93A MN/G93A A, even without addition of GLP-1, larger numbers of transgenic motoneurons in G93A MN/WT A system than of those cocultured with G93A astrocytes were counted, while this difference was not observed in the wildtype motoneuron co-culture systems. This may indicate a protective effect of wildtype astrocytes on transgenic motoneurons.

Further experiments and the assessment of different neurotoxic and neuroprotective compounds at different concentrations and in different co-culture conditions will be necessary. The motoneuron-astrocyte co-culture system could represent a valuable in vitro screening tool for novel neuroprotective compounds in ALS and provide more detailed insights into neuron-glia interaction in ALS.

### Literature

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The GLP-1 analog used was Acetyl-GLP-1-(7-34)-amide from Curatis Pharma GmbH, Hannover, Germany