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Neuroprotective effects of *Rhodiola rosea* extracts against excitotoxicity and oxygen-glucose deprivation in hippocampal slice cultures

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Introduction

The medical plant *Rhodiola rosea* (roseroot, golden root) is known as a stimulant of mental and physical endurance, increasing resistance to chemical, biological, psychological and physical stressors (Panossian et al. 2010). Extracts of *R. rosea* roots contain flavonoids, phenolic acids, phenylethanol derivatives (e.g. salidroside) and phenylpropanoid glycosides (e.g. rosavin) (Ioset et al. 2011). Many of these compounds are considered potent antioxidants with putative neuroprotective potential (e.g. salidroside (Shi et al. 2011)), but the significance of the various substances for the beneficial effects of Roseroot is still largely unknown. Here we tested the neuroprotective effects of crude methanolic extracts of *R. rosea* as well as chemical fractions and/or purified compounds (e.g. salidrosid, rosavin) against excitotoxicity and ischemia-like brain damage using organotypic hippocampal slice cultures.

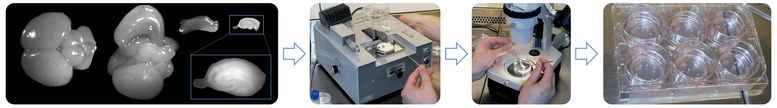

Figure 1. Hippocampal slice culture method

Materials & Methods

Crude methanolic extracts of R. rosea roots and flowers (Clone 5, Pharmaplant, Germany, grown for four years in our horticulture facilities), as well as chemical fractions of this extract (Table 1) were prepared and partly analysed by LC-MS.

Hippocampal slice cultures derived from 8 days old rat pups were grown at 33°C in serum-optimum for 2-3 weeks before exposure to N-methyl-D-aspartate (NMDA, 10 µM, 24 or 48 h) or oxygen-glucose deprivation (OGD, 30 or 35 min) at 36°C (Noraberg et al. 2005), with and without MK801, *Rhodiola rosea* extracts or single consitutuents (e.g. rosavin, salidroside) before (24 h), during (35 min) and/or immediately after the insult (for 48 h).

NMDA- or OGD-induced neuronal cell death was quantified by propidium iodide uptake and immunohistochemical staining of MAP2 as a neuronal marker.

Statistics: *) p<0.05, **)p<0.01 ,***p<0.001 One–way ANOVA with Benferroni's multiple comparison test versus NMDA or OGD (day 1 and day 2)

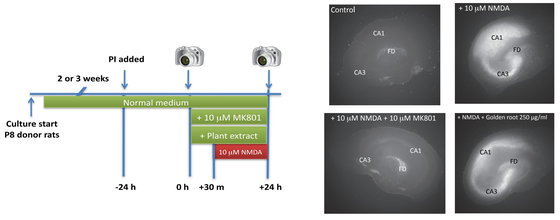

Figure 2. Experimental protocol and propidium iodide (PI) uptake method for quantifying NMDA or OGD-induced cell death and neuroprotection by MK801 or *R. rosea* extract.

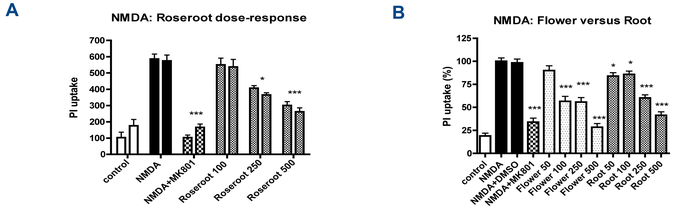

Figure 3. A. Cell death in CA1 quantified by PI uptake at day 1 (first bar) and day 2 (second bar) after NMDA treatment (10 µM) and protection by co-treatment with MK801 or Roseroot (root) extracts (for protocol see figure 2). N=14-18 cultures for each group.
B. Comparison of flower versus root extracts assessed at day 1 after treatments. N=16-41 for each group.

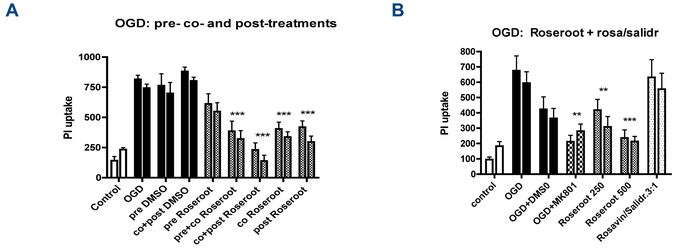

Figure 4. A. Cell death in CA1 quantified by PI uptake at day 1 (first bar) and day 2 (second bar) after OGD treatment (35 min) and protection by pre, co- and/or post-treatment with Roseroot (root) extracts N= 10 for controls, 28 for OGD, 12-17 cultures for Roseroot treatments.
B. Protection against OGD by MK801 (N=13) and Roseroot extract (N=7-8), but not by rosavin (3 µM) / salidroside (1 µM) (N=5). N= 13 for controls, 10 for OGD, 14 for OGD+DMSO.

Table 1. Testing fractions of Rhodiola rosea extracts*

Fraction	% of whole extract	Compounds present	Dose range tested	Neuroprotection in NMDA model	Neuroprotection in OGD model
A	?	Flavonoids	1-100 µg/ml, BUT: in-soluble in medium	Not known	Not known
B	3.0%	Not known	1-100 µg/ml	No	
C	4.3%	Not known	1-100 µg/ml	No	
D	3.5%	Not known	1-100 µg/ml	No	
E	5.4%	Not known	2-200 µg/ml	No	
F	5.3%	Rosavin	2-200 µg/ml	No	
G	2.6%	Rosavin	1-100 µg/ml	No	
H	76.5%	Salidroside, Tyrosol	Not tested yet	Not known	Not known
Pooled fractions B-G	Ca. 24%		20-100 µg/ml	No	No
Single compound		Rosavin	10-100 µM	No	
Single compound		Salidroside	10-100 µM	No	

* The methanol extract was subjected to bioassay-guided chromatographic fractionation by flash column chromatography using a dichloromethane–methanol gradient resulting in 8 fractions (A–H). Extract and fractions were analyzed by LC-MS for identification of the bioactive compounds in the different fractions.

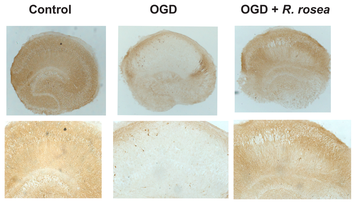

Figure 5. Representative MAP2 immunohistochemical staining of cryostat-cut sections (20 µM) of hippocampal slice cultures of controls, OGD and OGD + Roseroot extract (250 µg/ml) co-treated cultures, fixated 24 h after OGD.

Results

Significant, dose-dependent protection against NMDA and OGD-induced CA1 pyramidal cell death was obtained by crude methanolic extracts of Roseroot (roots or flowers) using 250 µg/ml (33-50% protection) or 500 µg/ml (45-65% protection) (Figures 3, 4, 5).
A number of chemical fractions of methanolic Rhodiola extracts, as well as the purified constituents salidrosid and rosavin were tested, but – so far – none of the tested fractions or single constituents showed protection against NMDA or OGD (Table 1).

Conclusion and perspectives

Methanolic extracts of Rhodiola rosea provide potent neuroprotection against excitotoxic (NMDA) or ischemic (OGD) cell death in hippocampal slice cultures.

The active compounds are probably found in fractions A and/or H (table 1), which will be further characterized by LC-MS and re-tested in slice cultures.

We are currently analyzing micro array microRNA and gene analyses data of Roseroot treated cultures and peforming Western blotting for selected proteins.

References

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Acknowledgement

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