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

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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* (rosenoot, 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 *Rh. rosea* roots contain flavonoids, phenolic acids, phenethylamides derivatives (e.g. salidroside) and phenylpropanoid glycosides (e.g. rosavin) (Isset 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 *Rh. rosea* as well as chemical fractions and/or purified compounds (e.g. salidroside) against excitotoxicity and ischemia-like brain damage using organotypic hippocampal slice cultures.

**Materials & Methods**

Crude methanolic extracts of *R. rosea* roots and flowers (Clane 5, Pharmaplet, 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 pups were grown at 35°C in serum-optimum for 2-3 weeks before exposure to N-methyl-D-aspartate (NMDA, 10, 20 or 40 µM) or oxygen-glucose deprivation (OGD, 30 or 35 min) at 32°C (Norberg et al. 2005), with and without MK801. *Rhodiola* rosea extracts or single constituents (e.g. rosavin, salidroside) before (24 h), during (35 min) and/or immediately after the insult (for 48 h) to the serum-supplemented cultures (pre, co- and/or post-treatment with Roseroot (root) extracts).

**Experimental protocol** and propidium iodide (PI) uptake method for quantifying NMDA or OGD-induced cell death and neuroprotection by MK801 or *Roseroot* (root) extracts (for protocol see figure 2). N=14-18 cultures for each group.

**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 *Roseroot* extracts, as well as the purified constituents salidroside 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 *R. 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 performing Western blotting for selected proteins.

**References**


**Acknowledgement**

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Table 1. Testing fractions of *Rhodiola rosea* extracts

<table>
<thead>
<tr>
<th>Fraction</th>
<th>OGD protection</th>
<th>NMDA protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>50±1.0</td>
<td>29±1.0</td>
</tr>
<tr>
<td>B</td>
<td>55±0.9</td>
<td>27±1.2</td>
</tr>
<tr>
<td>C</td>
<td>45±0.8</td>
<td>25±1.2</td>
</tr>
<tr>
<td>D</td>
<td>40±0.9</td>
<td>25±1.2</td>
</tr>
<tr>
<td>E</td>
<td>40±1.0</td>
<td>20±1.2</td>
</tr>
<tr>
<td>F</td>
<td>35±0.8</td>
<td>15±1.2</td>
</tr>
<tr>
<td>G</td>
<td>30±1.0</td>
<td>10±1.2</td>
</tr>
<tr>
<td>H</td>
<td>35±0.9</td>
<td>5±1.2</td>
</tr>
</tbody>
</table>

* The method was adapted to bioactive-aided chromatographic fractionation by fast column chromatography using a diol/triaminomethyl-graphite 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 3. A. Cell death in CA1 quantified by PI uptake at day 1 (first bar) and day 2 (second bar) after NMDA treatment (100 µM) and protection by MK801 (10 µM) or Roseroot extract (250 µg/ml) pre- and post-treatment. N= 10 for controls, 28 for OGD, 11-17 cultures for Roseroot treatments. B. Comparison of flavonoid versus test extracts assessed at day 1 and after treatments. N=4–11 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 pretreatment with Roseroot root (250 µg/ml) vs. Roseroot root (250 µg/ml) pre- and post-treatment with Roseroot root (250 µg/ml). B. Protection against OGD by MK801 (10 µM) and Roseroot extract (10 µM) but not by rosavin (3 µM) or salidroside (1 µM). N= 10 for controls, 10 for OGD, N= 5 for ROSERROD.

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) cocultures, fixed 24 h after OGD.