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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 medicinal plant *Rhodiola rosea* (rosenroot or golden root) is known as a stimulant of mental and physical endurance, increasing resistance to chemical, biological, psychological and physical stressors (Pankoska et al. 2019). Extracts of *R. rosea* roots contain flavonoids, phenolic acids, phenylethanoid 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 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. salidroside) against excitotoxicity and ischemia-like brain damage using organotypic hippocampal slice cultures.

**Materials & Methods**

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

Organotypic hippocampal slice cultures derived from 8 days old rat pups were grown at 33°C for 10 days 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 (Norberg et al. 2005), with and without Roseroot, *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).

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

**Results**

Significant, dose-dependent protection against NMDA and OGD-induced CA1 pyramidal cell death was obtained with 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 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 *Rhodiola rosea* provide potent neuroprotection against excitotoxicity (NMDA) and 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 microarray microRNA and gene analyses data of Roseroot treated cultures and performing Western blotting for selected proteins.

**Acknowledgement**

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**References**


Norberg, J., Innanen, J., Arkle, T., Spranger, M., Wideråer, L., Gaidus, C., Norling, M., Mengel, M., Gramsbergen, J. B. and Zimmer, J. (2005) Organotypic hippocampal slice cultures derived from 8 days old rat pups were grown at 33°C for 10 days 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 (Norberg et al. 2005).


**Table 1.** Testing fractions of *Rhodiola rosea* extracts

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Neuroprotection against NMDA and OGD-induced cell death and neuroprotection by MK801 or Roseroot extracts</th>
<th>Neuroprotection against NMDA and OGD-induced cell death and neuroprotection by MK801 or Roseroot extracts</th>
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<tbody>
<tr>
<td>A</td>
<td>33%</td>
<td>33%</td>
</tr>
<tr>
<td>B</td>
<td>50%</td>
<td>50%</td>
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<tr>
<td>C</td>
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<tr>
<td>D</td>
<td>75%</td>
<td>75%</td>
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</tbody>
</table>

**Figure 1.** Hippocampal slice method

**Figure 2.** Experimental protocol and propidium iodide (PI) uptake method for quantifying NMDA or OGD-induced cell death and neuroprotection by MK801 or Roseroot 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 extract at 250 µg/ml in each of the three treatment groups. B. Comparison of fraction versus root extracts assessed at day 1 after treatments. N=4-6 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- or post-treatment with Roseroot (root) extract. N= 14 for controls, 28 for OGD. 101 cultures for Roseroot treatments. B. Protection against OGD by MK801 (H1) and Roseroot extract (H1) but not by rosavin (10 µM) or salidroside (10 µM). N= 14 for controls, 10 for OGD. M for ROSEROOT.

**Figure 5.** Representative MAP2 immunohistochemical staining of cryo-cut sections (25 µM) of hippocampal slice cultures of controls, OGD and OGD + Roseroot extract (250 µg/ml) control cultures, fixed 48 h after OGD.