Neuroprotective Activity of *Rosmarinus officinalis* L. Extract in an I*n Vitro* Model of Krabbe Disease

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Krabbe disease is a lethal, progressive, autosomal recessive, neuro-degenerative disorder with no cure, characterized by the progressive demyelination and presence of globoid cells and thus, also called the 'globoid cell leukodystrophy'. In the most common infantile form of Krabbe disease, children appear normal at birth and present with neurological symptoms by six months of age. Clinical signs include irritability, neuropathy, sensory deficits and seizure. Krabbe disease is caused by a genetic deficiency of lysosomal hydrolase, galactosylceramide ß-galactosidase (GALC). In the absence of GALC, psychosine (D-galactosyl-sphingosine), a major component of myelin, accumulates to high levels in the central nervous system (CNS) and primarly in oligodendrocytes (OLs). Psychosine is generated by galactosylation of sphingosine by UDP-galactose: ceramide galactosyltransferase (CGT), a galactosylceramide synthesizing enzyme which is primarily expressed in OLs. Psychosine is a highly cytotoxic lipid, and its accumulation is presumed to be the cause of oligodendrocyte apoptosis in Krabbe disease. Several approaches for the treatment of this neuro-degenerative disorder are targets of research, but current treatment opinions for the disease are very limited. Gene therapy might be effective; however, a stable and strong expression vector is required (Pannuzzo et al., 2010). Some studies reported that brains from patients with Krabbe disease and twitcher mice show expression of the inflammatory cytokine TNF-a and IL-6, and established the relationship of psychosine and cytokine-induced cellular redox alterations by increase in reactive oxygen species (ROS) and the inhibition of peroxisomal functions (Pannuzzo et al., 2010).
Rosmarinus officinalis L. (Lamiaceae) is used as a folk medicine around the world. In medicine, the extract is receiving increasing attention due to its anti-inflammatory and antioxidative constituents (Russo et al., 2009). There are several reports that have established carnosic acid as the major phenolic diterpenoid present in *R. officinalis* leaves with antioxidant activity (Russo et al., 2009). In this study, we investigated the *in vitro* effect of a *R. officinalis* methanolic extract, containing 39.7% of carnosic acid, against psychosine-induced damage in cultured oligodendrocyte progenitor mice cells (OLP-II). The results showed a time-dependent (24-48h) and concentration-dependent (25-50 μM) decrease of OLP-II viability (MTT Test) and an increase in nuclear DNA damage (COMET assay) after exposure to psychosine and a dose-dependent (0.5-5 μg/ml) protection with the examined extract. They also evidenced that the extract treatment reduced the expression levels of apoptosis proteins p53, Bax, activated caspase-3, cleaved poly (ADP-ribose) polymerase 1 (PARP-1), and TNF-related apoptosis-inducing ligand (TRAIL), and increased the levels of the antiapoptotic protein Bcl-2, as compared with the levels in cultures treated with psychosine alone. Taken together, these preliminary data, indicating that *R. officinalis* methanolic extract protects against psychosine-induced apoptosis in OLP-II, suggest that an integration with this natural product may constitute a promising clinical management of the late-infantile and juvenile forms of Krabbe disease, and encourage further studies to examine its potential protective effect *in vivo*.

Pannuzzo et al. (2010). *Mol Genet Metab*. 100, 234-240.
Russo et al. (2009). *Natural Prod. Commun.* 4, 1707-10.