The neuroprotective versus neurotoxic properties of SALSOLINOL and its enantiomers

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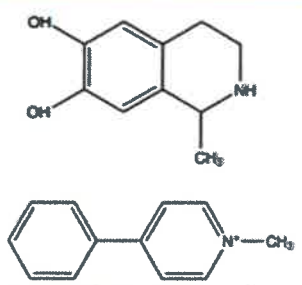

Figure 1. Salsolinol (top) and MPP+ (bottom).

BACKGROUND & AIM

Salsolinol (SAL, 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline), since its first detection in the urine of Parkinsonian patients treated with L-DOPA, has been proposed as a possible neurotoxic contributor to the disease. While MPTP and its metabolite 1-methyl-4-phenylpyridinum ion (MPP^+) are well recognised dopaminergic neurotoxins. However, SAL might also possess neuroprotective properties due to the presence of catechol moiety. Previously, we confirmed its antioxidant and neuroprotective properties in vitro (lactate dehydrogenase test as well as MTS, ROS, and caspase activity assays). The aim of the present study was therefore to purify SAL enantiomers and to compare the neuroproptective properties of *R*-SAL, *S*-SAL and the racemate in vitro.

MATERIALS AND METHODS

R,S-SAL was purified by means of HPLC with retention time 17.058 min and 21.575 min for *S*-SAL and *R*-SAL, respectively. SH-SY5Y cells were seeded at a concentration of 2.5×10^4 cells/well and cultured for 24 h to reach 70% confluence. Cells were preincubated for 1 h with either *R,S*-SAL or enantiomers and next either MPP^+ (1000 µM) or H_2O_2 (350 µM) was added. After 24-48 h of incubation, the MTS assay was used for the measurement of cells viability.

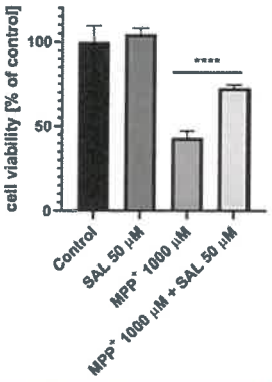

Figure 2. MTS test showing the neuroprotective effect of 50 µM *R,S*-SAL on SH-SY5Y neuroblastoma cells viability damaged by 1000 µM of MPP^+ after 48 h of incubation. Statistical significance set at ****$p < 0.001$ in comparison with the positive control 1000 µM MPP^+.

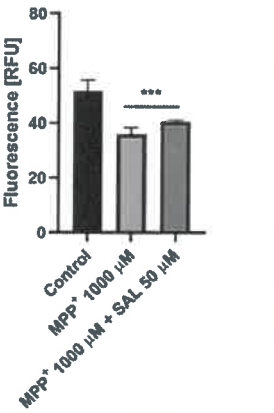

Figure 3. Neuprotective effects of *R,S*-SAL on loss of mitochondrial membrane potential (MMP^+). MMP^+ was monitored using rhodamine 123, a cell permeable cationic fluorescent dye that preferentially partitions into mitochondria based on the highly negative MMP^+. The fluorescence intensity was measured by fluorescence microscope Leica DMi8. Statistical significance set at ***$p < 0.001$ in comparison with the positive control 1000 µM MPP^+.

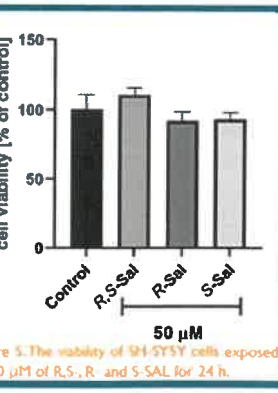

Figure 5. The viability of SH-SY5Y cells exposed to 50 µM of *R,S*-, *R*- and *S*-SAL for 24 h.

RESULTS

The eluates contained *S*-SAL with less than 0.1% of *R*-SAL as well as *R*-SAL with about 4% of *S*-SAL, were aliquoted, lyophilized, and stored in dark microtubes. The amount of the purified SAL enantiomers was further checked spectrophotometrically. Cell viability was significantly increased in SH-SY5Y cells exposed to a mixture of *R,S*-SAL (50 µM) and MPP^+ (1000 µM) in comparison to MPP+ alone as well as exposed to a mixture of *R*- or *S*-SAL (50 µM) and H_2O_2 (350 µM) in comparison to H_2O_2 alone. Yet, SH-SY5Y neuroblastoma cells' viability was indifferent between *R,S*-SAL and its enantiomers at the concentration of 50 µM, and was similar to the negative control.

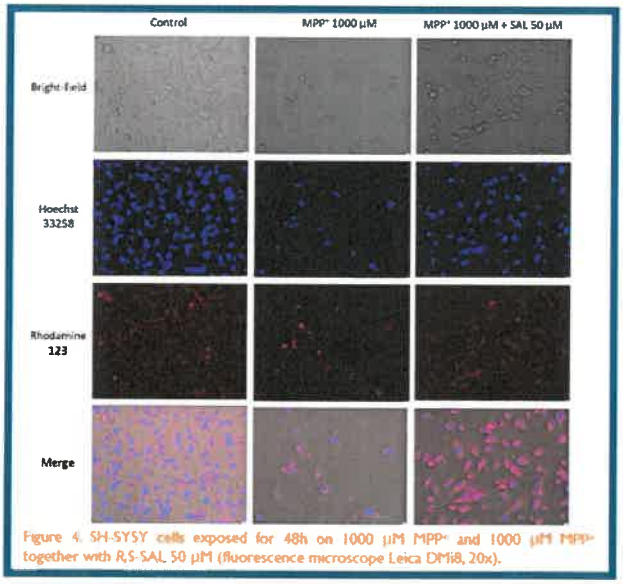

Figure 4. SH-SY5Y cells exposed for 48h on 1000 µM MPP^+ and 1000 µM MPP^+ together with *R,S*-SAL 50 µM (fluorescence microscope Leica DMi8, 20x).

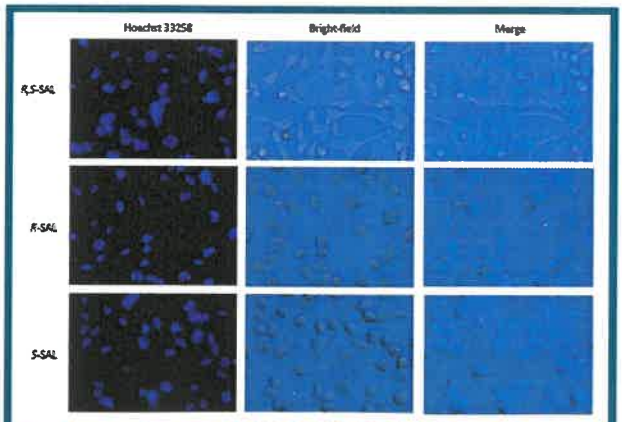

Figure 6. SH-SY5Y cells exposed to 50 µM of *R,S*-, *R*- and *S*-SAL for 24 h (fluorescence microscope Leica DMi8, 40x).

CONCLUSIONS

Our data suggest that possible neuroprotective role of SAL may not necessarily be related to stereoselectivity and confirm that *R,S*-SAL and its enantiomers are non-toxic at low doses.

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