A121 Solubilization of lichen metabolites in non-toxic solvents for tissue culture testing

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Poor solubility has often impeded pharmacological investigations of compounds isolated from lichens, e.g. testing for growth inhibitory effects on mammalian cells in culture. Although solvents such as DMSO, ethanol, or even tetrahydrofuran can be resorted to in specific assays, they are not suitable as versatile solubilizers for bioactivity testing. Two lichen metabolites, atranorin and (+)-usnic acid, were chosen as prototypes for solubilization studies on the basis of poor solubility, chemical character and ease of isolation in sufficient quantities. The aim was to find solvents capable of meeting two set criteria, i.e. capacity to solubilize the compounds and lack of direct activity on a chosen test cell line, the human leukemia K-562 cell line, in a standard proliferation assay. Solubilization was measured at different pH values in various concentrations of cosolvents (glycofurol, propylene glycol, polyethylene glycol 400, phenoxyethanol), surfactants (polysorbate 20, polysorbate 80, Cremophor RH40®,Cremophor EL®) and the complexing agent 2-hydroxypropyl-β-cyclodextrin. For solubility determination, HPLC analyses of saturated solutions of lichen compounds in the relevant solvents were performed. Three of the solvents proved suitable for tissue culture use in terms of non-toxicity, i.e. propylene glycol, PEG 400 and 2-hydroxypropyl-β-cyclodextrin. Of these, best results for solubilizing (+)-usnic acid were obtained using 10% 2-hydroxypropyl-β-cyclodextrin, giving a maximum usnic acid concentration of 0.35 mg/ml. Best results for solubilizing atranorin were obtained using concentrated PEG-400, resulting in a maximum concentration of 0.37 mg/ml. When tested against the K-562 cell line, atranorin proved inactive at the maximum obtainable concentration while (+)-usnic acid exhibited significant activity, with an ED_{50} value of 4.7 µg/ml. Further antiproliferative testing of (+)-usnic acid in 10% 2-hydroxypropyl -β-cyclodextrin against the breast cancer cell line T-47D gave comparable results, with an ED_{50} value of 4.2 µg/ml.

A122 The effect of lichen polysaccharides on rat spleen cell proliferation *in vitro*

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Many polysaccharides isolated from lichens and fungi have been found to have immunomodulating and/or antitumour activities (1,2). The aim of this study was to investigate the effect of homogene lichen polysaccharides with well-defined structures, on spleen cell proliferation *in vitro*.
Polysaccharides of different structural types (1) were selected for the study: isolichenan and lichenan from *Cetraria islandica*, nigeran from *Stereocaulon alpina* thamnolan and Ths-2 (3) from *Thamnolia vermicularis* var. *subuliformis*, a galactomannan from *Peltigera canina* with Man/Gal/Glc ratio (65:29:6), and pustulan, a (1→6)-β-glucan which is O3-acetylated on 17% of the units in the backbone, isolated from *Umbilicaria proboscidea*. The polysaccharides were extracted and purified as described before (4), and structurally characterized by ^{1}H and ^{13}C NMR spectroscopy, metanolysis and methylation analysis using GC-MS. Cell proliferation *in vitro* was performed with spleen cells from rats that were either treated with Ths-2 or thamnolan s.c. or saline as a negative control. The cells were cultured for 24 hours in the presence of different concentrations of all the above mentioned polysaccharides. The proliferation was determined as ^{3}H-thymidine uptake in dividing cells using scintillation counter. Results from the proliferation assay using spleen cells from thamnolan or saline rats showed that isolichenan and the galactomannan stimulate proliferation with the highest stimulation index (SI) of 2.3-2.5 for isolichenan and 1.9-2.0 for the galactomannan at concentration of 167 µg/ml. Surprisingly, pre-treatment of the rats with Ths-2 resulted in reduced spleen cell proliferation when stimulated with isolichenan or the galactomannan (SI 1.8 or 1.4, respectively). Since each study group contained only 3 rats, this difference did not reach a significant level. No indication of stimulation of spleen cell proliferation *in vitro* could be detected for nigeran, pustulan, lichenan, Ths-2 or thamnolan.

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