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The novel slow-releasing hydrogen sulfide (H2S) donor P* induces apoptotic cell death in Jurkat leukemia T cells

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Background & Aim: Numerous studies have reported about the different role of H2S in cell survival, proliferation and apoptosis in human cancer cell lines. In the present work, we investigated the anti-proliferative and pro-apoptotic potential of substance P* in Jurkat leukemia T cells. P* is a persulfide analog of the nitrosothiol S-nitroso-N-acetyl-D,L-penicillamine (SNAP) which releases H2S only in the presence of thiols (reduced glutathione or L-cysteine).

Materials & Methods: Proliferation and cell viability of Jurkat T cells were determined with 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide assay, Annexin-V/7-AAD staining and Western blot. To induce cytokine production, the cells were stimulated with phorbol 12-myristate 13-acetate plus A23187 with or without different concentrations of P*. The release of interleukin (IL-) 2 was quantified by enzyme-linked immunosorbent assay.

Results: Substance P* reduced proliferation and cell viability of Jurkat T cells in a dose-dependent manner. P* induced cleavage of pro-caspase-3/-7, poly (ADP-ribose) polymerase, myeloid cell leukemia-1 and β-catenin. The addition of high concentrations of antioxidants such as N-acetyl-cysteine or L-cysteine could completely prevent apoptotic cell death of Jurkat T cells. In strong contrast to P*, the "classic" H2S donor sodium hydrogen sulfide (NaHS) did not affect cell viability. Besides the pro-apoptotic activities of P*, it also blocked IL-2 synthesis.

Conclusion: The slow-releasing and thiol-inducible H2S donor P* showed potent antiproliferative and pro-apoptotic activities in Jurkat leukemia T cells. We suggest that treatment of Jurkat T cells with P* may lead to an imbalance in the redox system (GSH depletion?) which in turn induces the apoptotic (caspase-3) signaling pathway.

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A high throughput solubility assay by microscale shake-flask and rapid quantification of UHPLC-UV-CLND for drug discovery

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The rapid determination of key physical properties of lead compounds is essential to the drug discovery process. Solubility is one of the most important properties since good solubility is needed not only for obtaining reliable *in vitro* and *in vivo* assay results in early discovery but also to ensure sufficient concentration of the drug being in circulation to get the desired therapeutic exposure at the target of interest. In order for medicinal chemists to tune solubility of lead compounds, a rapid assay is needed to provide solubility data that is accurate and predictive so that it can be reliably used for designing the next generation of compounds with improved properties. To ensure speed and data quality, we developed a high throughput solubility assay that utilizes a single calibration UHPLC-UV-CLND method and a 24 hours shake-flask format for rapid quantification. A set of 46 model compounds was used to demonstrate that the method is accurate, reproducible and predictive. Here we present development of the assay, including evaluation of quantification method, filtration membranes, equilibrium times, DMSO concentrations and buffer conditions. A comparison of thermodynamic solubility results to our high throughput 24 hour shake-flask solubility assay results is also discussed.

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