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In vitro and in vivo distribution of vorinostat, a nonselective inhibitor of histone deacetylase, in different blood compartments

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Suberoylanilide hydroxylamic acid (SAHA, vorinostat) as the first histone deacetylase (HDAC) inhibitor approved by the FDA has attracted great interest and the positive results from clinical trials yield hope to use vorinostat as a potential anti-tumour agent. Little is known about the relationship between extracellular (plasma) concentration and intracellular (PBMCs) concentration and therefore the potential impact of pharmacokinetic variability and different dosing regimens on efficacy. It is thus important to correlate the relationship between HDAC enzyme activity and concentration, a question which was addressed in our study.

Several highly sensitive and selective analytical methods using HPLC coupled to electrospray tandem mass spectrometry were developed to quantify the concentrations of vorinostat and its two metabolites simultaneously. These methods were validated in different matrices according to the regulations of FDA. BD Vacutainer® CPT™ tube was used for PBMC isolation. We developed a liquid-liquid extraction method to extract vorinostat from plasma and PBMCs but had to develop an SPE method to extract vorinostat and its more polar two metabolites. Extracts were chromatographed on a Phenomenex® Luna C18 column with isocratic for plasma and gradient for PBMCs which were consisting of acetonitrile, methanol and 5 mM ammonium acetate including 0.1% aqueous acetic acid and 0.1% acetonitrile. Vorinostat and its two metabolites were quantified by using their deuterated compounds as internal standards. The calibration curve for plasma vorinostat ranged from 10-1000 ng/mL with a LOQ of 10 ng/mL. The corresponding values for vorinostat in PBMCs were 0.1-10 ng/3 x 10^6 cells with a LOQ of 0.1 ng/mL. The calibration curve of vorinostat/ M1/ M2 in

plasma ranged from 11/11/11-1100.4/ 11000/ 11000 ng/mL with LOQ is 11/11/11 ng/mL. The correlation coefficients were always $r^2 \geq 0.999$ (linear regression) and appropriate accuracy and precision was obtained. Over all matrices, the within-batch accuracies varied between 88.95% and 111.4% and the batch-to-batch accuracies ranged from 90.92% to 109.5%. From plasma the recoveries of vorinostat were 75.38% (low), 84.94% (medium), and 83.97% (high). For co-extraction of vorinostat and its two metabolites the recoveries of vorinostat/ M1/ M2 were 106.2%/ 88.6%/ 108.2% (low), 95.7%/ 94.9%/ 98.2% (medium), and 97.2%/ 98.7%/ 103.3% (high). The respective values in PBMCs were 114.7% (low), 93.05% (medium), and 96.98% (high).

HDAC activity was quantified with a fluorogenic enzymatic assay and it correlated with HDAC inhibitor concentration in a sigmoid manner. When applied to samples from healthy volunteers an enrichment of vorinostat in the PBMCs was found which was most pronounced at lower plasma concentrations. When the same methods were applied to blood samples of tumour patients at steady-state also in their PBMCs vorinostat accumulation was observed albeit accumulation appeared less pronounced. Additionally we also applied the methods to different solid tumour cell lines in vitro and did not find a similar enrichment of active compound, which might indicate that cellular access is regulated differently in different cells. This is a rather important finding because it may point to a mechanism determining susceptibility of the cells.

Our methods were successfully applied to in vitro and in vivo studies. The stability results showed that the plasma and PBMCs samples that were stored either at RT in 72 h or at -20/-80 °C for around one year are stable. This will give advantages for collecting patient samples. The results showed that the distribution of vorinostat in different blood compartments is uniform but the mechanism controlling access of vorinostat to the target cells is still unknown. Future studies should focus on factors potentially affecting vorinostat distribution to blood compartments and tissue and determine the metabolism of vorinostat in PBMCs in vivo and tumour cells in vitro.