Surfaced-enhanced Raman spectroscopy (SERS) as an innovative pharmacological tool to quantify thiopurine metabolites in immortalized human hepatocytes (IHH).

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Thiopurines are antimetabolite drugs with antiblastic and immunosuppressive actions used to treat leukemia but also autoimmune disorders and after organ transplants. Azathioprine (AZA), mercaptopurine (MP) and thioguanine (TG) are inactive prodrugs that require enzymatic transformation to cytotoxic thioguanine nucleotides (TGNs) in order to carry out their actions. Despite the proven therapeutic efficacy of these drugs, significative dose-dependent adverse effects on bone marrow and liver but also idiosyncratic toxicity on the pancreas are reported. Monitoring of thiopurine metabolite levels in patients can be helpful for their correct clinical use. Currently, several efficient methods are available to quantify thiopurine metabolites including high-performance liquid chromatography and mass spectrometry. However, these techniques require relatively sophisticated sample processing and analysis. Surface-enhanced Raman spectroscopy (SERS) is an inexpensive and rapid technique that enhances, through gold and silver nanoparticles, the weak Raman scattering, correlating vibrational modes with molecular structures.

Therefore, the aim of this work was to set up a simple, rapid and relative inexpensive method to quantify thiopurine metabolites in human immortalized hepatic (IHH) cell lysates with SERS. This cell line is representative of the tissue mainly involved in thiopurine biotransformation after oral administration and target of dose-dependent thiopurine toxicity.

Initially, we focused on the study of SERS spectra generated by IHH cell lysates in order to avoid possible interferences of biomolecules in the subsequent thiopurine analysis. SERS spectra were obtained by a Raman ReniShaw spectrometer using Ag and Au colloidal nanoparticles as SERS substrates. Lysates were prepared sonicating cells in sodium citrate buffer (10 mM pH=4.5). Intense SERS spectra of IHH lysates were observed only after filtration with Amicon Ultra devices (cut-off 10 KDa). Moreover, SERS spectra were different depending on the metal nanoparticles. Representative bands were generated mainly by the vibrational modes of adenine, adenosine and reduced form of glutathione (GSH). In particular, in SERS spectra of IHH lysates obtained with Au substrates three characteristic bands at 732 cm-1, 659 cm-1 and 1316 cm-1 were found and attributed to adenine, guanine and adenosine respectively. For analysis performed with Ag nanoparticles the main contribution of the SERS spectra was attributed to GSH with the exception of 728 cm-1 band attributed to adenine.

Moreover, preliminary SERS analysis on thiopurines and their metabolites in buffer and lysate solutions were performed resulting in intense and repeatable spectra, mostly with Au substrates. In addition, SERS spectra of IHH cells treated with AZA and MP for 48 and 96 hours were acquired. Data obtained showed the presence of several bands ascribable to thiopurines supporting an association between SERS spectra intensity and concentration of the compounds. Consequentely,

two predictive models used to quantify MP and thioinosine monophosphate (TIMP), an important metabolite of thiopurines, in IHH cells treated with AZA and MP for 48 and 96 hours were built. The models highlighted a complete transformation of MP into thiopurine metabolites and a decrease of TIMP concentration at 96 hours respect to 48 hours. In particular, in cells treated with MP and AZA for 48 hours TIMP concentration was 310±11 nM/106 cells and 191±20 nM/106 cells respectively while, at 96 hours of treatment the concentration decreased to 161±5 nM/106 cells for MP treatment and to 24±4 nM/106 cells for AZA treatment. This work demonstrated the possibility to carry out qualitative and quantitative analysis of thiopurine metabolites by SERS in a complex biological matrix, such as hepatocytes, considering in perspective for quantitative data further validation by reference methods such as mass spectrometry.