Development of an LC-MS/MS-based method for quantification Tdp1 inhibitor based on usnic acid and distribution study in tissue of healthy and tumor mice

Alina A. Okhina[a,b], Tatyana E. Kornienko[c], Artem D. Rogachev[a,b], Olga A. Luzina[a], Nelly A. Popova[b,d], Valery P. Nikolin[d], Alexandra L. Zakharenko[c,], Nadezhda S. Dyrkheeva[c], Andrey G. Pokrovsky[b], Nariman F. Salakhutdinov[a] and Olga I. Lavrik[b,c]

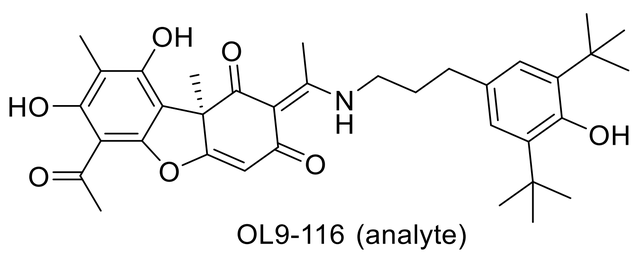
[a]N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry of the Siberian Branch of Russian Academy of Sciences, Lavrent'ev ave., 9, Novosibirsk, 630090, Russia
[b]Novosibirsk State University, Pirogov St. 2, Novosibirsk, 630090, Russia
[c]Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of Russian Academy of Sciences, 630090, Lavrent'ev ave., 8, Novosibirsk, Russia
[d]Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Lavrent'ev ave., 10, Novosibirsk, 630090, Russia

Introduction

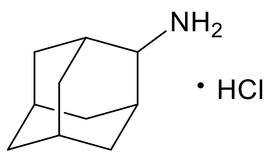
Tyrosyl DNA phosphodiesterase 1 (Tdp1) is a promising target for the treatment of cancer [1], since it plays a key role in the removal of DNA damage generated by the action of topoisomerase 1 (Top1) poisons (irinotecan and topotecan) widely used in anticancer therapy [2]. Tdp1 is a key enzyme that is involved in Top1cc repair. Tdp1 is considered one of the reasons for the lack of effectiveness of Top1 poisons in the treatment of cancer and, in turn, as a target for additional therapy that can increase the effectiveness of Top1 poisons [3]. Thus, the development of prodrugs Tdp1 inhibitors combined with Top1 poisons is an actual task.

We have previously shown that the Tdp1 inhibitor, enamine derivative of usnic acid, the agent OL9-116, enhances the antitumor activity of topotecan, which resulted in a decrease in metastases and tumor size [4]. We also studied the pharmacokinetics of the agent and the obtained results helped us to improve the effectiveness of Tpc anticancer therapy *in vivo* [5].

The target organ in the treatment of Lewis carcinoma is the lungs. When drugs administered intragastrically, they can partially be metabolized in the liver before entering the systemic circulation. In this regard, the purpose of this work is to develop and validate an LC-MS/MS methods for the quantification of OL9-116 in mouse lungs, liver, kidneys, Lewis lung carcinoma tumor nodes and studied its distribution in organs of healthy and tumor mice.



OL9-116 (analyte)



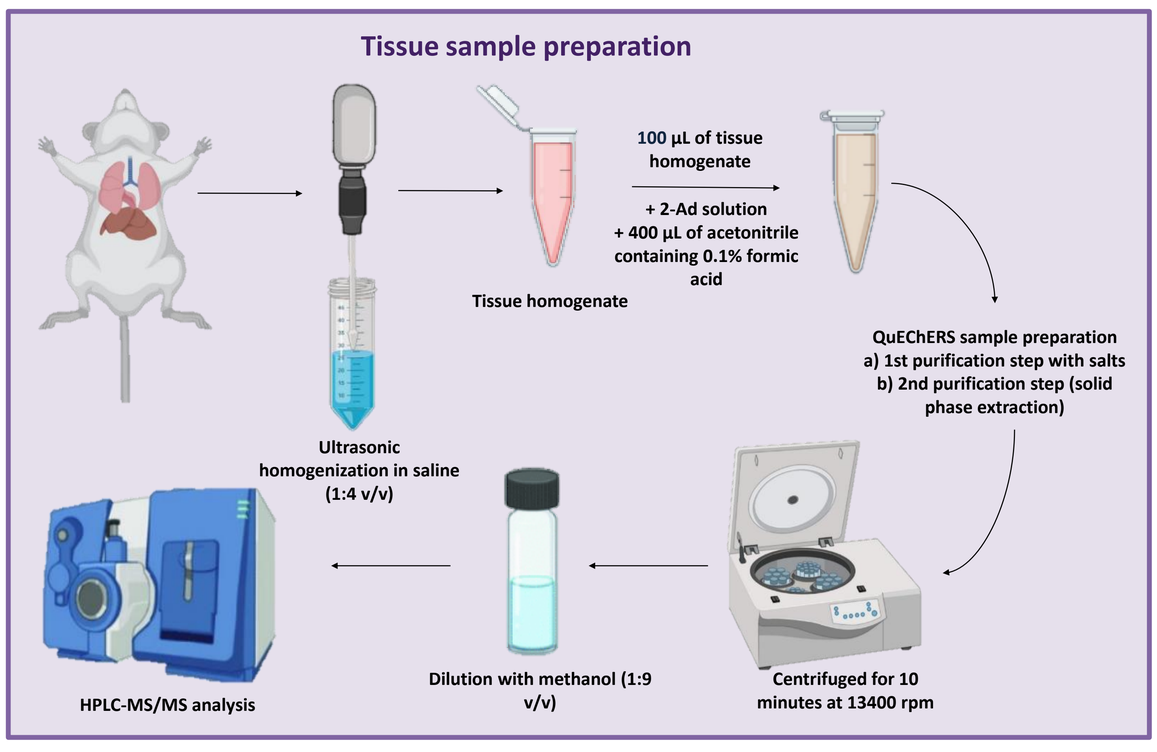
2-adamantylamine hydrochloride (IS)

Materials and methods

The substance OL9-116 was synthesized earlier in our laboratory and had purity over 95% according to NMR data. The internal standard (IS) was a solution of 2-adamantylamine hydrochloride (2-Ad) in acetonitrile with a concentration of 20 µg/mL for processing tissue samples.

The experimental tumor was transplantable Lewis lung adenocarcinoma (LLC). This cancer cell line was obtained from the cell depository of the Institute of Cytology and Genetics (Novosibirsk, Russia) and was maintained in mice as a transplanted tumor. Prior to the transplantation, the tumor tissue was crushed and resuspended in 0.9% NaCl solution. Tumor cells were inoculated intramuscularly into the right thigh (0.1 ml volume; 800 000 tumor cells per mouse); a primary tumor node was formed at the injection site.

Mice were administered OL9-116 150 mg/kg intragastrically on day 17 after transplantation of tumor cells. There were three mice for each time point. The lungs, kidneys, liver and tumor were taken from mice euthanized by the method of cervical dislocation 30 minutes, 1, 3, 7, 13 hours after administration of the substance. The extracted organs were thoroughly washed with 0.9% saline (NaCl).



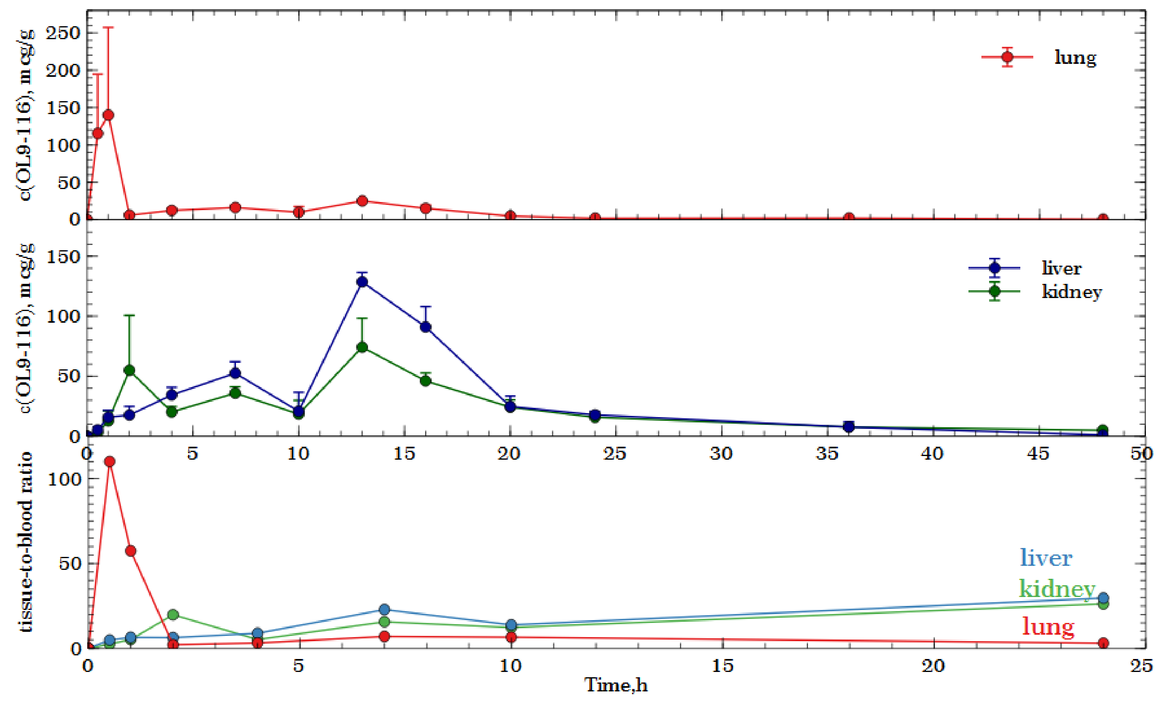
Validation of the developed methods were performed in accordance to regulatory documents of the FDA and EMA for the following parameters: selectivity, calibration curve, accuracy, precision, recovery, matrix factor, and stability of the analyte in the prepared sample.

Results and discussion

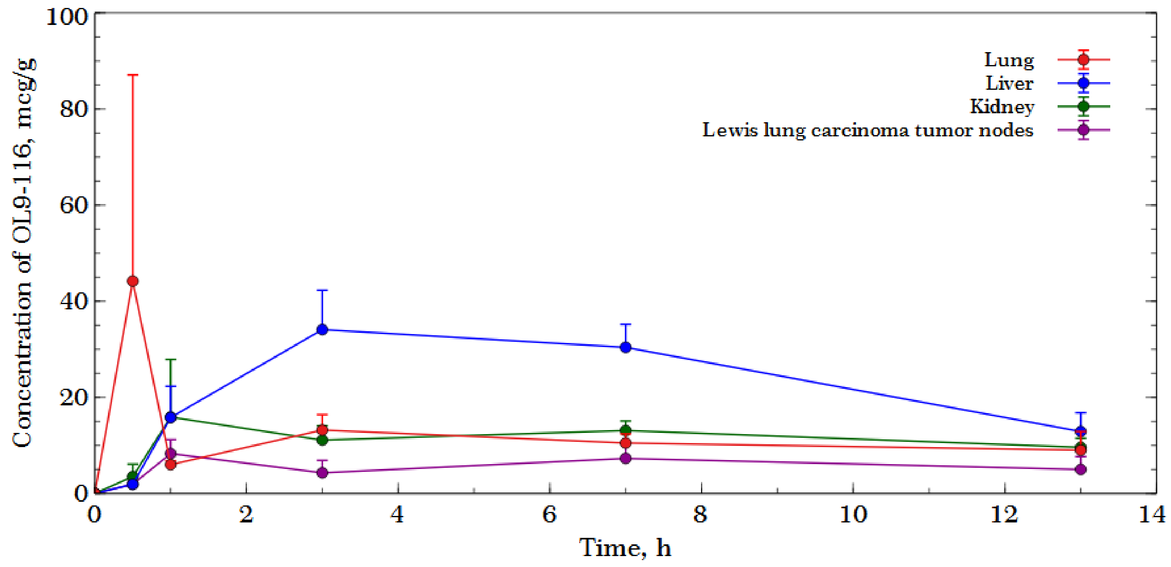
In our study, we developed a fast, selective and sensitive procedure for quantification of OL9-116 in mouse tissue using HPLC-MS/MS. OL9-116 distribution in organs was studied on healthy and tumor mice. It was found that maximal concentration of the agent in organs of healthy mice can reach 100 µg/g followed by its distribution and excretion. The maximum concentration in the lungs was reached after 1 hour and is approximately 140 µg/g. The concentration value was several mg/g for one animal at the 30-minute point, which lies above the calibration range and is an outlier in relation to the other two points. The large scatter of SEM is due to the small number of animals participating in the experiment and the nature of the tissue.

We observed two maximum concentrations studying pharmacokinetics after 7 and 13 hours in the liver, 2 and 13 hours in the kidneys. We assume that the presence of multiple maxima is a result of the effects of liver enzymes.

We would like to note that the full pharmacokinetics investigation of the compound in the organs would require using and sacrificing a large number of animals: to obtain a full statistics for a time point it is necessary to get six or more data values for this point.



In tumor mice we observed increasing concentration of OL9-116 during first 1 h after administration to a level of 5-30 µg/g depending on the tissue (lungs, liver, kidneys, tumor node) and remaining at this level for at least next 12 hours, thus showing a pharmacokinetics profile clearly differing from that of healthy animals.



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