Abstract (thesis):

Palmar-plantar erythrodysesthesia (PPE) frequently accompanies the therapy with a continuous 5-FU infusion or peroral capecitabine (5-FU prodrug). In the most severe cases this adverse effect leads to discontinuation of a needful therapy. Local 10 % uridine ointment is used to prevent and treat the said adverse event. Nevertheless, this method is not generally accepted as an effective one because it has never been proved in a randomized controlled clinical trial. Most probably, a direct effect of a cytostatic compound on the skin of hands and foots causes PPE. The toxicity of 5-FU is mediated primarily by its incorporation into RNA and by thymidylate synthase (TS) inhibition and subsequent DNA synthesis disruption. The importance of particular 5-FU toxicity mechanisms varies in different cell types. For choosing the best PPE local antidote it is necessary to find out which molecular mechanism applies in keratinocytes.

We have chosen pyrimidine nucleosides as the most suitable compounds for the local PPE therapy because the uridine ointment is already being used in several oncology centers in the Central Europe. In order to find out the 5-FU toxicity mechanism, we further tested the effect of calciumfolinate (CF) which strengthens the TS inhibition by 5-FU. We studied also uracil and pyridoxine as potential 5-FU antidotes. We tested *in vitro* the individual compound potential to prevent toxic 5-FU effects on the HaCaT cell line of spontaneously immortalized human keratinocytes. We used three different and complementary methods of cellular viability testing: microscopical assessment of the evolution of morphological changes documented in microphotographs, metabolic activity assessment by means of the MTT test and measuring the cellular adherence within time by the RTCA test.

We confirmed that uridine significantly prolongs the cellular survival in the 5-FU presence. We also confirmed that cytidine protects the cells to the same extent. Uridine prevents 5-FU incorporation into RNA and thus we confirmed that this mechanism is crucial for the 5-FU toxicity in the HaCaT keratinocytes. When we added thymidine to uridine, further increase in metabolic activity and prolongation of cellular survival occurred. This effect was rather synergic than additive as thymidine solely did not prolong the cellular survival at all. This means that thymidine prolongs cellular survival by abrogating TS inhibition only when the 5-

FU incorporation into RNA is blocked by uridine. We confirmed this hypothesis also by experiments with calciumfolinate (CF). CF increased the 5-FU toxicity only when the 5-FU incorporation into RNA was blocked by uridine. CF did not increase the 5-FU toxicity when no protective compound was added. Therefore uridine “switches” the 5-FU toxicity mechanism from RNA incorporation to TS inhibition which can further be strengthened by CF. When 5-FU, uridine and thymidine were present in the cell culture medium, the 5-FU toxicity was not increased by CF. This confirms the fact that TS inhibition by 5-FU is abrogated by thymidine and therefore CF cannot be effective as a 5-FU toxicity booster. Cytidine showed the same protective effect as uridine probably after its metabolisation to uridine. Uracil showed only a significantly lower protective effect in comparison with uridine. Deoxycytidine and pyridoxine did not prevent the 5-FU toxicity at all.

We confirmed that the 10 % uridine ointment application in PPE treatment and prevention can be supported by *in vitro* results of the uridine 5-FU antidotal effect in the HaCaT cells. This effect was further confirmed by several experiments on primary keratinocytes. From the theoretical point of view, cytidine would be similarly effective as uridine when placed into the ointment but the lower stability of this nucleoside (cold storing requirement) makes its clinical employment difficult. The combination of uridine with thymidine for further clinical testing is advisable so that the protective ointment efficacy could be increased.

Key words: Palmar-plantar erythrodysesthesia, Hand-foot syndrome, 5-fluorouracil, uridine, cytidine, thymidine