Abstract

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Title of diploma thesis: The effect of the proteasome inhibition of the antiproliferative effect of anthracycline antibiotics

Anthracycline antibiotics (daunorubicin, doxorubicin) belong to the most effective antitumor drugs. In current clinical practice they are used mostly in the combinations with either "classical" or new targeted antitumor drugs. The proteasome inhibitors (bortezomib and carfilzomib) are also viewed as a part of new "targeted" antitumor drugs. The proteasome is a multienzyme complex in eukaryotic cells which is responsible for intracellular degradation of proteins. The proteasome inhibitors have been largely used in the therapy of multiple myeloma, but their potential has been also studied in the case of other malignancies. Their use in the combination with anthracyclines could be a possible alternative in the therapy of some tumor illnesses, but the effect of combination of anthracyclines and proteasome inhibitors on tumor cells have not been sufficiently explained. The anthracycline therapy is also accompanied by serious adverse side effect – the cardiotoxicity, which potential could be influenced by the combination with proteasome inhibitors.

The main aim of this master thesis was the evaluation of the antiproliferative activity of proteasome inhibitors (bortezomib and carfilzomib) on human promyelocytic leukemia cells (HL-60) and the influence of these drugs on the antiproliferative effect of daunorubicin on these tumor cells. The next goal was also to study the influence of these drugs on the toxicity of daunorubicin in *in vitro* anthracycline cardiotoxicity model – isolated rat's neonatal ventricular cardiomyocytes.

The antiproliferative activity of proteasome inhibitors was tested on suspension cell culture HL-60. The cell viability was evaluated by MTT test after 72 hours of the incubation of cells with daunorubicin, doxorubicin, bortezomib and carfilzomib in a wide range of

concentrations. IC_{50} of all studied drugs was calculated from these data. After that the combination effect of drugs was analyzed using Chou-Talalay method. Bortezomib and carfilzomib showed a quiet profound antiproliferative effects on leukemia cell culture with IC_{50} values in nmol/dm^3 unit order. The combination with anthracyclines did not lead to the significant increase of antiproliferative effect (the combination index was in wide range of concentration scale higher than 1).

The influence of proteasome inhibition by bortezomib and carfilzomib on the viability of isolated neonatal cardiomyocytes was evaluated after 48 hours of the incubation of cells with tested compounds. It was measured the activity of lactate dehydrogenase released from cells to the cultivating medium during the incubation. The values of IC_{50} for bortezomib and carfilzomib were determined. The influence of proteasome inhibition on daunorubicin cardiotoxicity was evaluated on that model. It was found that the proteasome inhibition in our scheme does not significantly influence the toxicity of daunorubicin towards primary neonatal cardiomyocytes. This observation must be supplemented also by other evaluations in the next time schemes and concentrations of bortezomib and carfilzomib.