Abstract

Neuroblastoma is the most common extracranial solid tumor of childhood. Despite advances in cancer diagnosis and therapy, the treatment of some forms of neuroblastoma is still complicated. One of the major complications of the chemotherapy is a developed drug resistance.

This master thesis deals with the effect of cytostatics on protein and gene expression of selected proteins, which may contribute to chemoresistance of the human neuroblastoma cell line UKF-NB-4. The sensitive line UKF-NB-4 and the resistant line UKF-NB-4^{CDDP}, UKF-NB-4^{DOXO} and UKF-NB-4^{ELLI} were exposed to cisplatin, doxorubicin, ellipticine for 24, 48 and 72 hours.

The Western blot analysis showed that cytostatic agents cisplatin, doxorubicin or ellipticine added to the sensitive neuroblastoma cell line UKF-NB-4 in amounts which are added to resistant neuroblastoma cell lines in order to maintain resistance induced expression of p53 and reduced expression of retinoblastoma protein pRb after 72 hours of cultivation. Differences in the expression of RAS protein, cytochrome P450 1A1, 3A4 and cytochrome b_5 has not been shown. Changes in the expression of the studied proteins in resistant lines UKF-NB-4^{CDDP}, UKF-NB-4^{DOXO} and UKF-NB-4^{ELLI} cultured with and without cytostatic agents were not detected by the Western blot analysis.

Lower level of the p53 gene was detected in the sensitive neuroblastoma cell line UKF-NB-4 after 72 hour cultivation with cisplatin or doxorubicine using RT-PCR while no effect of ellipticine on the p53 gene was detected. Conversely, cultivation of neuroblastoma cell lines resistant to cisplatin with cisplatin increased the level of the p53 gene. The gene expression of pRb after 48 hours cultivation of the sensitive neuroblastoma line UKF-NB-4 with cytostatics corresponded to the pRb levels after 72 hours cultivation of the cell line with cytostatics detected by the Western blot analysis. Lower levels of the H-RAS and cytochrome P450 2D6 gene expression were detected in the UKF-NB-4 cell line cultivated with cisplatin or doxorubicin for 48 hours, however there was no difference in the expression of the RAS protein and cytochrome P450 2D6 was not detected by the Western blot.

The knowledge of the mechanisms responsible for chemoresistance at gene and protein level is crucial for cancer treatment.

Keywords: neuroblastoma, tissue culture, Western blot, RT-PCR, cisplatin, doxorubicin, ellipticine, p53, pRb, RAS, cytochrome P450, cytochrome b_5 **(In Czech)**