Summary

The possibilities of current therapy of cancer diseases are wide, yet these diseases are still among the most common causes of death. The current findings of biomedical research are increasingly being applied in the treatment strategy of cancer. Understanding the molecular pathways associated with cancer cell formation, progression, and metastasis has led to the discovery of new anticancer drugs and the development of targeted therapies. Simultaneously, knowledge of the molecular mechanism of action of anticancer drugs derived from plants or their semisynthetic derivatives makes it possible to expand the portfolio of cytostatics used so far.

The aim of the dissertation was to study the cytotoxic effect of hitherto unexplored montanine-type isoquinoline alkaloids *in vitro* using a panel of cancer cell lines and a non-cancer cell line of human lung fibroblasts.

In the initial phase of the study, the basic types of montanine alkaloids, montanine and pancracine, were isolated, further subjected to cytotoxicity screening using a panel of 9 cancer cell lines of different histotype and one non-cancerous cell line formed by lung fibroblasts. Mean inhibitory concentrations IC_{50} values were determined. Pancracine, the parent alkaloid of the Amaryllidaceae family, has been comprehensively studied in order to understand the molecular mechanism of its action, especially the effect on viability and proliferation of the resistant lung adenocarcinoma cell line A549 and the leukemic cell line MOLT-4. As part of a more detailed study of the molecular mechanisms of action, methods have also been used that have shown its effect on cell cycle, replication, induction of apoptosis, and detection of molecular pathway proteins leading to antiproliferative and cytotoxic effects. First, cell proliferation and cancer cell viability were measured by Trypan blue staining assay and xCELLigence real-time proliferation system. The effect on the cell cycle was determined by flow cytometry. Apoptosis was determined by Annexin V / PI labelling and quantification of caspase activity (-3/7, -8 and -9). Proteins involved in growth arrest or apoptosis activation were detected by electrophoresis and western blotting. Pancracine significantly reduced the viability and proliferation of the leukemic cell line MOLT-4. The apoptosis-inducing effect of pancracine in MOLT-4 cells was demonstrated by a significantly higher caspase activity as well as by the detection of phosphatidylserine on the outer leaflet of the plasma membrane of leukemia cells. Further evidence of apoptosis is the detection of the p53 tumor suppressor protein phosphorylated at serine 392, the proapoptotic MAP kinase p38 phosphorylated at threonine 180, and tyrosine 182, and the upregulation of the inhibitor of cyclin-dependent kinases p27. Pancracine significantly inhibited the proliferation of the A549 lung adenocarcinoma cell line and this effect persisted for 96 h. Growth inhibition of resistant lung adenocarcinoma was due to increased cell accumulation in the G1 phase, this G1 block was caused by downregulation of the tumor suppressor protein Rb phosphorylated at serine 807 and 811, upregulation of p27, and downregulation of Akt kinase phosphorylated at threonine 308.

In conclusion, cell cycle perturbation and induction of apoptotic cell death were considered to be the key mechanisms of pancracine action.