Pharmacological approaches to ameliorate vincristine neuropathy
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Vincristine has been an important agent in the treatment of especially pediatric malignancies for four decades. The dose-limiting side effect of vincristine is peripheral neuropathy resulting in pain, constipation, paresthesias, and impaired motor function. Besides this neuropathy, other side effects are relatively mild and no serious long-term side effects are known, such as second malignancies or infertility. At present, we still don't know how to prevent or overcome vincristine neuropathy other than by reducing or interrupting therapy, which might lead to a less optimal oncolytic effect. Because of its efficacy and relatively mild non-neurotoxic side effects, it is worthwhile to try to maximize its use as an anticancer agent. Finding ways to ameliorate vincristine neuropathy would therefore be of great interest.

In this thesis two pharmacological approaches are described to achieve this goal. First, we hypothesized that neuropathy might be related to vincristine pharmacokinetics; a higher degree of neuropathy might correlate with a higher systemic exposure. Therefore, we studied vincristine pharmacokinetics and its relation to neuropathy in children. Second, we studied recombinant human insulin-like growth factor-I as a potential neuroprotective agent against vincristine induced neuropathy in a rat model and its potential adverse effects on tumor growth and the antitumor effect of vincristine in cell lines and a xenograft mouse model.

Background information about vincristine is given in chapter 1. Experimental studies are described in chapters 2 through 6.

In chapter 1 we review knowledge about vincristine, indicate where knowledge is lacking, and suggest potential future research areas to enhance its oncolytic effect and reduce its side effects. Vincristine is an alkaloid, extracted from the periwinkle plant Catharanthus roseus. It has a broad oncolytic spectrum and high response rates as single agent and since the early nineteen sixties it has been incorporated in multidrug protocols for the treatment of especially pediatric hematological and solid malignancies.

Its dose-limiting side effect is a peripheral, sensory-motor and autonomic neuropathy, resulting in pain, loss of deep tendon reflexes, constipation, and sometimes paresthesias, sensory loss and motor weakness, the syndrome of inappropriate antidiuretic hormone, or cranial nerve dysfunction. The degree of neuropathy is variable and largely unpredictable. While formerly neuropathy was thought to be totally reversible in recent studies long-term sequelae are recognized. Other side effects
are relatively mild, like myelosuppression, which makes it easy to combine with other chemotherapy. Vincristine appears not to induce second malignancies nor lead to infertility in humans.

Vincristine's most widely known mechanism of action is disruption of microtubules function. In dividing cells microtubules form the structural elements of the mitotic spindle and in nerve fibers they are involved in axoplasmic transport needed for maintenance of the axon. Damage to microtubules of the mitotic spindle by vincristine results in mitotic arrest of cells, leading to its oncolytic effect. Damage to microtubules in nerve fibers results in axonal degeneration, leading to peripheral neuropathy. Knowledge about induction of apoptosis and anti-angiogenesis as mechanisms of action (and maybe also pathophysiology of neuropathy) is just emerging. New insights in these aspects might lead to new strategies to optimize the oncolytic effect of vincristine.

Knowledge about vincristine pharmacokinetics, metabolism and the relation between pharmacokinetics and the oncolytic effect or neurotoxicity is largely missing. Information about the relationship between vincristine pharmacokinetics and pharmacodynamics might contribute to the design of dosage regimens with better oncolytic effect and less neurotoxicity.

In vitro, several mechanisms of vincristine resistance are recognized, as multidrug resistance and tubulin changes; their clinical importance is less clear. Effective and safe ways to modulate vincristine resistance, and thus optimize its oncolytic effect are still under investigation.

Agents studied to protect against vincristine neuropathy include folinic acid, vitamin B1, B6, isaxonine, glutamic acid, gangliosides, and the ACTH analog ORG 2766. Most of these agents were unsuccessful in patients. Recombinant human insulin-like growth factor I, nerve growth factor, and amifostine seem to be new promising agents, which need further study to evaluate their neuroprotective effect.

Experimental studies dealing with vincristine pharmacokinetics and its relation to neuropathy are described in chapter 2 through 4. Chapters 5 and 6 deal with the potential neuroprotective and adverse effects of recombinant human insulin-like growth factor-I.

Chapter 2 describes the high performance liquid chromatography (HPLC) assay to measure vincristine concentrations in plasma and refinements in the assay
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made to reduce the required sample volume. These make the assay more suitable for use in small children. The reduction of the required sample volume was realized by changing the material of the extraction column, and using a more sensitive and controlled electrochemical detector. These refinements resulted in a reduction of the sample volume from 1.2 ml to 0.3 ml plasma. A very low limit of quantitation of 0.48 ng/ml according to good laboratory practice rules could be preserved.

Chapter 3 concerns a descriptive pharmacokinetic study. Intrapatient and interpatient variability in vincristine disposition and demographic, clinical, and biochemical characteristics influencing this variability in children are described. Children were treated for acute lymphoblastic leukemia or non-Hodgkin's lymphoma according to the Dutch Childhood Leukemia Study Group protocol ALL8 and Wilms' tumor according to the National Wilms' Tumor Study protocol IV. Vincristine dosage was 1.5 mg/m² administered weekly as iv bolus injection. Pharmacokinetics were studied after 169 vincristine injections in 32 patients. Plasma concentrations were measured by HPLC. A two compartment first order model was used to fit to the data and pharmacokinetic parameters were calculated from this model using the ADAPT II software. Besides sex, age, and diagnosis, co-medication, liver-, and kidney-function, total plasma protein, albumin concentration, and platelet count were monitored. Although results show a larger interpatient variability than intrapatient variability in elimination half-life, total body clearance, apparent volume of distribution, and area under the concentration time curve, both were large. Children treated for acute leukemia and non-Hodgkin's lymphoma had a significantly higher total body clearance than children treated for Wilms' tumor. Variability however, remained largely unpredictable.

In chapter 4 the relationship between vincristine pharmacokinetics and clinical neuropathy in children with acute lymphoblastic leukemia is described. Children were treated according to the Dutch Childhood Leukemia Study Group Protocol ALL8. Pharmacokinetics of the first four weekly vincristine injections were studied in the same way as described for chapter 3. Pharmacokinetics were studied after each of 52 vincristine injections in 13 patients. Degree of clinical neuropathy was scored at baseline and one week after the fourth vincristine injection according to a modified WHO scoring system. 1 Minute vincristine peak concentration, 24 hours plasma concentration and area under the concentration time curve were related to increase in WHO score. A higher 1 minute peak vincristine concentration correlated with a higher
degree of clinical neuropathy. This correlation was determined by the correlation between peak concentration and gastrointestinal autonomic neuropathy, not sensory motor neuropathy.

In **chapter 5** we studied the protective effect of recombinant human insulin-like growth factor-I (rhIGF-I) on vincristine induced sensory motor neuropathy in rats in a placebo controlled design. Neuropathy was assessed by scoring gait disturbance, measuring tail-flick latency time, and rota-rod-performance in the light and in the dark. Neuropathy was scored at baseline, during and upon completion of the 2 week study period. We found that rhIGF-I ameliorated vincristine induced neuropathy. RhIGF-I delayed vincristine induced gait disturbance and protected against vincristine induced decreased rota-rod performance in the dark, but did not protect against vincristine induced increased tail flick latency time. These results suggest that rhIGF-I protects against proprioceptive sensory damage, but not against thermal noxious sensory damage. The protective effect against motor neuropathy could not be definitely answered in this study.

In **chapter 6** the potential adverse influence of rhIGF-I on cell growth and cytotoxicity of drugs is described in a preclinical setting. We studied the influence of rhIGF-I on cell growth and the antitumor effect of vincristine in human rhabdomyosarcoma cell lines under serum-free and serum-containing conditions. We found that under serum-containing conditions, in contrast to the less physiological serum-free conditions, rhIGF-I did not have a significant influence on cell growth. We could not find a negative influence of rhIGF-I on the antitumor effect of vincristine. To extend the in vitro data with a model which has more similarity with patients we studied the same issues in a childhood rhabdomyosarcoma xenograft mouse model. Results found in vitro were confirmed in vivo.

We conclude that vincristine pharmacokinetics are variable between and within patients and that tumor type or treatment protocol influences part of this variability: ALL patients have a higher clearance than Wilms' tumor patients. A higher vincristine peak concentration correlates with a worse gastrointestinal autonomic neuropathy in children with acute lymphoblastic leukemia during remission induction treatment. RhIGF-I was found to ameliorate vincristine induced gait disturbance, and decreased rota-rod performance in the dark in rats, suggesting that it protects against vincristine induced proprioceptive sensory neuropathy. We did not find an adverse effect of
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rhIGF-I on tumor growth and the antitumor effect of vincristine in cell lines and xenograft models.

In summary, results of the research presented in this thesis indicate that vincristine induced gastrointestinal autonomic neuropathy might be ameliorated by avoiding high peak concentrations and that sensory neuropathy might be ameliorated by using rhIGF-I in children. Avoiding high peak vincristine concentrations might be achieved by giving infusions instead of intravenous bolus injections. Obviously, we first need to know the relationship between vincristine pharmacokinetics and the intended, oncolytic effect. Studies to describe this relationship are presently undertaken. RhIGF-I should be tested in a phase I/II and III study to determine its side effects, optimal dosage regimen, and neuroprotective effect in children. If we are able to reduce neuropathy, dose intensity of vincristine can be increased to maximize its oncolytic effect.