The cytotoxic drug cyclo-pentenyl cytosine: from manufacturing to anti-tumor activity and (cardio)toxicity

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CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

INTRODUCTION

The agent cyclopentenyl cytosine (CPEC) is a cytidine analogue with potential anti-tumor activity. Contrary to the newest targeted drugs, CPEC is not involved in specific intracellular signalling pathways or angiogenesis, but ultimately leads to inhibition of DNA synthesis and therefore could be classified as a classic cytostatic drug [1]. In this thesis, pharmaceutical aspects as well as anti-tumor activity and mechanisms of cardiotoxicity of CPEC are explored.

CPEC

CPEC can be considered as a prodrug; after transmembrane transport the drug is phosphorylated to form its active metabolite CPEC-TP (cyclopentenyl cytosine triphosphate). CPEC-TP inhibits the enzyme CTP synthetase (cytidine triphosphate synthetase) which is involved in the *de novo* synthesis of CTP out of UTP (uridine triphosphate). CTP can also be formed from the salvage pathway by phosphorylation of cytidine. However, several malignancies seem to have a preference for the formation out of UTP. Inhibition of CTP synthetase may result in depletion of CTP pools leading to a decrease of RNA and DNA synthesis and S-phase accumulation. Several *in vitro* and *in vivo* studies have shown activity of CPEC against leukemia, neuroblastoma and colorectal cancer [2-4]. During a phase I study in patients with solid tumors severe cardiotoxic effects was found [5]. However, based on the studies that showed promising results on hematological malignancies, plans for phase I and II clinical trials (under cardiovascular monitoring) were initiated.

DEVELOPMENT OF CPEC FOR CLINICAL USE

CPEC is only available as a raw substance and was kindly provided by the National Cancer Institute (Bethesda, Maryland) in the U.S.A. Formulation is an important issue during the preclinical development of a drug. Aspects that need to be considered with regard to drug formulation: stability, reconstitution and safety upon handling. These last two characteristics are especially important for cytostatic drugs as they often form a potential risk upon occupational exposure. As described in chapter 3, it is possible to formulate a sterile and stable solution of CPEC which can be easily administered.

Therapeutic drug monitoring (TDM) can be useful when there is a proven relationship between blood levels and either toxicity or efficacy. Whether CPEC levels in humans are associated with efficacy is yet unclear. However, in a phase I study [5] it was shown that high concentrations of CPEC seemed to be associated with cardiotoxic side effects. The consequences of this finding are that future trials will have to start with lower dosages and that monitoring of plasma levels

may be required. This also means that an assay enabling monitoring of relatively low levels of CPEC becomes essential. Using LC-MS/MS we were able to quantitatively determine low levels of CPEC (chapter 4). The deaminated metabolite of CPEC: cyclopentenyl uracil (CPEU) could also be measured, although with a lower sensitivity. This might result in undetectable concentrations of the metabolite in the lower dose levels as is demonstrated by our results of the analysis of plasma levels of the first patient receiving low dose CPEC. However, as CPEU has almost no cytotoxic effects and its concentration was not associated with cardiotoxicity in the phase I study, no consequences are expected for the application of the assay in TDM of CPEC.

EFFICACY

The first studies with CPEC mainly focus on its activities as an antiviral agent. However, only *in vitro* studies on the antiviral activity have been published and no results in animals or humans are as yet known. More research has been performed on the use of CPEC as an anti-tumor agent. Activity against several solid tumors as well as hematological malignancies has been demonstrated in *in vitro* and in animal studies [2,4,6]. Considering the promising results in lymphocytic and myeloid leukemia, and the hematotoxic side effects in humans with solid tumors, CPEC might be of use in the treatment of ALL. As shown by our results using a xenogeneic *in vivo* model for ALL, efficacy was associated with severe toxicity (chapter 5). In earlier studies an increase in life span upon CPEC treatment was demonstrated. There might be several explanations for the different results between our study and the earlier *in vivo* studies. First, as we continuously monitored leukemic progression and toxicity, we were able to determine the actual response during treatment instead of having death as the only evaluation point for efficacy and toxicity. Moreover, in our model leukemic progression could be excluded as a possible cause of death, allowing discrimination between activity and toxicity of therapy. Secondly, we started treatment from the moment that leukemic cells could be detected and not immediately after inoculation. The third explanation might be that we administrated human leukemic cells whereas earlier studies used cells of murine origin which might respond differently to CPEC.

In leukemia combination therapy is frequently used. Reasons for this strategy might be to create synergy between drugs by acting via multiple pathways or reduction of toxicities by lowering the dose of the individual drugs. Regarding CPEC, cytarabine seems to be a logical candidate for combination therapy as the uptake and therefore activity of cytarabine is regulated by feedback inhibition of deoxy-CTP (dCTP). As CPEC can deplete CTP and therefore dCTP, it might be responsible for enhanced activity of cytarabine. Our *in vitro* results suggest an additive but not synergistic effect of CPEC and cytarabine. Therefore we chose not to investigate the combination treatment of CPEC and cytarabine in our *in vivo* animal model for human ALL. However, even if no synergistic effect can be accomplished, it might be interesting to investigate whether a combination of cytarabine and low dose CPEC will reach similar efficacy when compared to the individual agents and their level of toxicity.

*CARDIO*TOXICITY

As survival and cure rates for cancer increase, reduction and management of side effects become more important. Cardiotoxicity is an important problem associated with current chemotherapeutic regimens (chapter 6). Moreover, this is not exclusively a problem of the classic cytotoxic drugs, as several studies report cardiotoxic side effects after treatment with the newer targeted drugs. The monoclonal antibody trastuzumab and recently also imatinib and sunitinib [7-9] are examples of newer cytostatic drugs associated with cardiotoxicity. As shown in the case of CPEC, cardiotoxic side effects might hamper the introduction of potentially interesting drugs. It would be interesting to know why in particular the heart is susceptible to toxic side effects and how these effects can be prevented or predicted.

Mechanism

When taking a better look at anthracycline induced cardiotoxicity, it becomes clear that these answers are not so easily found. Although extensive research has been undertaken to study anthracycline induced cardiotoxicity, the mechanism is not completely clarified. Reactive oxygen radicals are supposed to play an important role and it has been postulated that due to a low level of antioxidant enzymes (e.g. SOD), the heart would be more susceptible to the destructive action of these radicals [10]. Following this theory antioxidant therapy might be useful; however, until now there have been no antioxidants that have been able to yield good results against anthracycline induced cardiotoxicity. In the anti-oxidative theory iron plays an important role as it is believed to form a free radical complex with reduced anthracyclines. The iron chelator dexrazoxane can bind iron and thereby prevent or reduce the formation of the complex. Dexrazoxane has shown promising results in preventing cardiotoxicity and is currently the only agent approved for the prevention of anthracycline induced cardiotoxicity [11,12]. It was also suggested that apoptosis plays a role and indeed doxorubicin treated mice showed an increased apoptotic rate in their hearts [13].

When studying the cardiotoxic effects of CPEC, we used an anthracycline based approach in our attempts to clarify its cardiotoxic effects (chapter 7). There are a few objections to be made against this approach. First, CPEC does not have a structural relationship with anthracyclines and from that point of view a similarity in mechanism might not be logical. Secondly, the observed hypotension after treatment with CPEC is not a common cardiotoxic side effect seen with anthracyclines. However, as anthracycline induced cardiotoxicity has been known for years, many models have been developed and validated to study this phenomenon. These models might therefore also be suitable for studying the side effects of other drugs. Moreover, in our animal model we studied a general phenomenon (apoptosis) that is also associated with CPEC treatment. We have not been able to detect CPEC induced apoptosis in rats. It is possible that some physiological

aspects of rats might have played a role in the absence of cardiotoxicity of CPEC in our study. Rats are reported to have very low levels of cytidine deaminase, the enzyme responsible for the deamination of cytidine to uridine. This might lead to high levels of cytidine which have been suggested to protect against CPEC induced toxicity. However, cytidine deaminase is also responsible for the deamination of CPEC to CPEU, and it is not known what ratio of CPEC-cytidine levels is necessary for efficacy or toxicity. Moreover, although we did not study it as extensively as in rats, we also could not demonstrate signs of cardiotoxicity in mice, which are reported to have cytidine deaminase activities comparable to humans [14,15].

Prevention and prediction

In current practice restricting the total cumulative dose, reducing peak levels, delivering the anthracycline in liposomes or administration of a protective agent (dexrazoxane) are the most effective tools to prevent cardiotoxicity. Although dose restriction might be easy to undertake, it is not always desirable and there are still patients (up to 5%) with cardiotoxicity despite the administration of lower doses, as well as patients that do not encounter any problems even after very high dosage of anthracyclines.

Moreover, the measures currently taken all seem to merely attempt to lower the total exposure of the drug to heart cells without understanding differences in individual sensitivity. It therefore becomes more important to be able to predict which patients are prone to chemotherapy induced cardiotoxicity.

The individual differences might indicate that there is a role for pharmacogenetics in chemotherapy induced cardiotoxicity. The study by Wojnowski *et al* is the first study to report an association between single nucleotide polymorphisms (SNPs) and anthracycline induced cardiotoxicity [16]. In this study a candidate gene approach is used and the investigators report an association with cardiotoxicity for six SNPs in five genes. The associated SNPs are located in genes involved in either NAD(P)H oxidase (p22phox, p40phox, Rac2) or doxorubicin efflux transporters (MRP1, MPR2). As described in chapter 8 we have demonstrated that the expression of MRP1 in rat cardiomyocyte cells decreased by doxorubicine and the expression of MRP2 showed a small decrease after incubation with CPEC. No changes in expression were observed for the other genes studied. Information on the influence of doxorubicin or CPEC on expression of the candidate genes might help in understanding the role of the gene and the specific SNP and their possible association with chemotherapy induced cardiotoxicity. Furthermore we have been able to reproduce the association between a polymorphism in the GTPase Rac2 which is essential for the function of the NAD(P)H oxidase multi-enzyme complex and cardiotoxicity of doxorubicin in a case control study. Sample size might have been an important reason for the absence of an association for the other five SNPs in our study. However, this is also an illustration of an important problem

for the clinical use of screening for these SNPs in anthracycline induced cardiotoxicity. Even if we would have been able to increase our sample size and could have reproduced the findings of the previous study, the frequency of overt anthracycline induced cardiotoxicity would still be low (1.1% in our study). Therefore, in order to identify patients with the associated SNPs, the 'number needed to genotype' would be unrealistically high. Moreover, as demonstrated by Wojnowski *et al* only 7-29% of the cases could be attributed to the carrier status of one of the candidate genes [16]. This implicates that knowledge of a cardiotoxicity associated SNP of a patient will only partly help in predicting whether this patient will ultimately develop anthracycline induced cardiotoxicity. Nevertheless, when combining genetic variants with other known risk factors, such algorithms or predictive models may prove a useful tool in the prediction of chemotherapy induced cardiotoxicity.

CONCLUSION AND FUTURE ASPECTS

In conclusion, the results presented in this thesis show that CPEC might not have fulfilled all of its promises. Single agent therapy in ALL proved to have a therapeutic window too small for further development. However, it cannot be ruled out that combination therapy in other hematological or solid tumors might be an useful addition to current therapeutic regimens. Positive findings are that in the studied animal models no further indications for CPEC induced cardiotoxicity were found. However, the results of the first study in patients experiencing severe hypotension cannot be neglected. This implicates that if CPEC is administrated in experimental protocols to patients, monitoring of cardiac function remains necessary. Unfortunately, cardiotoxicity is common among cytotoxic drugs and the vast amount of studies undertaken with anthracycline induced cardiotoxicity demonstrate that solutions to resolve the problem are not easily found. With the increasing possibilities of pharmacogenetics, a new modality might have become available. Although pharmacogenetics will not provide an absolute answer, combining our existing knowledge of risk factors with new genetic findings might bring us closer to good management of chemotherapy induced cardiotoxicity.

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