F, V & V in ObGyn, 2010, 2 (4): 278-286

Chemotherapy during pregnancy: pharmacokinetics and impact on foetal neurological development

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Abstract

Based on an estimated incidence of of 1 cancer case per 1000-1500 pregnancies, 3000-5000 new patients can be expected in Europe annually. The treatment of cancer in pregnant women is a challenge since both the maternal and the fetal well-being need to be considered. This study was initiated to gain more insight into the problems associated with cancer and chemotherapy during pregnancy.
A multicentric registration study was set up to evaluate the currently applied treatment modalities for cancer during pregnancy, and the consequences of their use for pregnancy. Secondly, a preclinical and clinical pharmacological study addressing pharmacokinetics of chemotherapy in pregnant women and transplacental passage of chemotherapy was performed. Thirdly, we investigated the effects of prenatal exposure to chemotherapy on fetal neurological development.
We observed an equal distribution of tumour types between pregnant and age-matched nonpregnant women. Data on neonatal outcome suggest that exposure to chemotherapy in the 2nd or 3rd trimester of pregnancy does not worsen outcome. This finding is explained by the fact that chemotherapy is not administered during the period of organogenesis and by the fetal protection offered by the placental barrier-function. Physiological changes of pregnancy resulted in a decreased plasma drug exposure of chemotherapeutic agents.
Before major conclusions can be drawn with regard to the long term fetal outcome and the efficacy of chemotherapy during pregnancy, more patients and a longer follow up period is required. Therefore, this research project is continued and expanded nationally and internationally.

Key words: Cancer, chemotherapy, pregnancy, pharmacokinetics, transplacental transfer.

Introduction

Cancer is the second leading cause of death in women during the reproductive years and complicates 0.06%-0.10% of pregnancies (Pavlidis, 2002). In Europe, yearly 3000 to 5000 patients are diagnosed with cancer during pregnancy. As women in developed societies defer childbearing to the third or fourth decade of life, and the incidence of several malignancies rises with increasing age, this relatively rare co-incidence is likely to become more common in the future.

The treatment of cancer in pregnant women is a challenge since both the maternal and the fetal well-being need to be considered. Clinical experience in cancer treatment during pregnancy is based on a rather limited compilation of case reports, small retrospective case series and reviews (Cardonick and Iacobucci, 2004; Pereg *et al*., 2008; Pentheroudakis and Pavlidis, 2006). In the absence of prospective studies related to cancer diagnosis and treatment during pregnancy, we initiated this study in 2005. In the first phase of the project, we focused on the current management modalities and the maternal and fetal outcome after cancer (treatment) during pregnancy. Furthermore, we initiated a pharmacological study addressing the pharmacokinetics of chemotherapy in pregnant women and their transplacental passage.

Table I. — Distribution of tumour types.

Tumour type	Number	Percentage
Breast cancer	99	46
Hematological malignancies	40	18
– Hodgkin Disease	13	6.0
– Non-hodgkin lymphoma	10	4.7
– ALL	4	1.9
– AML	7	3.2
– CML	4	1.9
– Hairy cell leukaemia	1	0.5
– Multiple myeloma	1	0.5
Dermatological malignancies	21	10
– Basal cell carcinoma	9	4.2
– Melanoma	11	5.1
– Kaposi sarcoma	1	0.5
Cervical cancer	17	8
Brain tumour	8	4
Ovarian cancer	8	4
Colorectal cancer	5	2
Other (sarcoma, lung, liver, kidney, GIST, thyroid, urachus, rhinopharyngeal)	17	8
	215	**100**

Finally, we investigated the effects of *in utero* exposure to chemotherapy on neurological development.

Current management modalities and maternal and fetal outcome (Van Calsteren *et al*., 2010a)

In order to assess current treatment modalities and the impact on the obstetrical and neonatal outcome, an international multicentric registration study was set up in Belgium, the Netherlands and the Czech Republic. Between 1998 and 2008, 215 patients with a diagnosis of cancer during pregnancy were registered. The most frequently encountered tumour types were breast cancer (46%), haematological (18%) and dermatological malignancies (10%) (Table I). These are the same types of tumours seen in nonpregnant women of this age group (Belgian Cancer Registry, 2008). This observation confirms the idea that pregnancy in itself is no risk factor for cancer.

In 5/215 (2.3%) patients, a miscarriage occurred at 10.7 ± 4.8 weeks of gestation, before cancer treatment was started. In 30/215 (14.0%) patients, the pregnancy was terminated at a gestational age of 10.9 ± 6.8 weeks; in 13 patients (43.3%), termination took place after 13 weeks gestational age. In 58/215 (27.0%) patients, treatment was deferred until postpartum; in these cases, the cancer diagnosis was made at a gestational age of 30.6 ± 9.4 weeks. In 122/215 (56.7%) patients, one or more treatment modalities were initiated during pregnancy, after a cancer diagnosis at a gestational age of 19.6 ± 8.5 weeks.

The mean gestational age at delivery was 36.2 ± 2.9 weeks. 8.4% delivered before 32 weeks (n = 15/179), 45.8% between 32 and 37 weeks of gestation (n = 82/179), and 45.8% at term (≥ 37 weeks) (n = 82/179). Thus, 54% of the children were born *preterm*, with a consequent high admission rate to the neonatal care unit. In the vast majority (90%) of patients, the delivery was induced. The complications of preterm birth are well-studied and include intraventricular haemorrhage, bradycardia/apnea, need for respiratory assistance, necrotising enterocolitis, sepsis, seizures, hypoglycaemia and feeding problems. Recently, Bastek *et al*. (2008) showed that late-preterm neonates (34-37 weeks) have significantly more medical complications compared to their term counterpart. Besides these immediate effects, preterm birth is associated with long-term morbidities and impaired cognitive and behavioural outcomes (Johnson, 2007). I believe that the consequences of preterm delivery in our study population are underestimated. The prevention of prematurity deserves our careful attention also in an oncological setting. Prevention of prematurity can be realised by postponing or continuing treatment until a term delivery can be obtained. Deliberate delay of therapy to achieve fetal maturity appears to be a safe option for certain patients with early-stage disease (Cold *et al*., 2005; Nettleton *et al*., 1996; Duggan *et al*., 1993). Continuation of treatment initiated during pregnancy is a alternative way to prevent prematurity. To date, treatment during pregnancy is continued until fetal viability is reached. Rather, fetal maturity should be the preferred criterion to induce labour. In a multidisciplinary setting, a maximal effort should be executed to delay delivery until at least 35-37 weeks.

Furthermore, our data show no increased incidence of *congenital malformations* after *in utero* exposure to chemotherapy in the 2nd or 3rd trimester of pregnancy. These observations confirm the evidence that cytotoxic treatment administered after the first trimester of pregnancy does not result in a higher incidence of congenital anomalies (Cardonick and Iacobucci, 2004; Aviles and Neri, 2001; Mir *et al*., 2008b).

Fetal *growth restriction* is a permanent concern when cancer is treated during pregnancy (Cardonick and Iacobucci, 2004; Fischer *et al*., 2006; Weisz *et al*., 2001), and was confirmed by the findings in the current study. In 26/175 cases, birthweight was below the 10th percentile for gestational age (14.9%, binomial test, p = 0.054). The low-birthweight cases were not related to a specific type of cancer, but the largest proportion of small-for-gestational-age newborns was documented in pregnancies with haematological tumours (9/33, 27.3%). Binomial testing revealed a significant increase of small-for-gestational-age neonates in the group receiving treatment during pregnancy (surgery, chemotherapy or radiotherapy) (n = 21/117 (17.9%). In mothers receiving chemotherapy and/or radiotherapy, small-for-gestational-age babies were seen in 16/66 (24.2%, binomial test p = 0.001), compared to 10/109 (9.2%) in the pregnancies without cytotoxic treatment (binomial test, p = 1.320). Whether the effect on fetal growth is determined by the maternal illness associated with malnutrition and a catabolic status (Nulman *et al*., 2001), or by direct or indirect effects of the treatment, remains an open question.

In conclusion, the findings of the current study show an overall good outcome of pregnancies complicated by cancer. However, a high rate of preterm inductions with a subsequent high rate of admissions to the neonatal care unit was observed. Interdisciplinary decision-making on the timing of delivery, with obstetricians and neonatologists, is necessary. Preferably, delivery should not be induced before 35-37 weeks. Current data confirm that cytotoxic treatment, administered during the second and third trimester of pregnancy, does not increase the rate of congenital malformations.

2. Pharmacokinetics of chemotherapy during pregnancy

In the second part of this research, we addressed the pharmacokinetics and transplacental passage of chemotherapy during pregnancy. Currently available information is based mainly on anecdotal reports and theoretical inferences from the physicochemical characteristics of the drugs, the general distribution characteristics of the drugs and the physiological adaptations in pregnancy (Wiebe and Sipila, 1994).

To the best of our knowledge, this study is the first to report on experimental research on the pharmacokinetic characteristics of chemotherapeutic drugs during pregnancy. The use of an animal model was necessary since experiments on transplacental transfer would be ethically unjustified in humans.

2A. Transplacental passage of chemotherapy

Transplacental transfer of drugs occurs primarily by passive diffusion. Therefore, the amount and rate of transfer is determined by the concentration-gradient of the drug between the maternal and fetal circulation and placental blood flow. Besides the physicochemical properties of drugs such as lipid solubility and ionization constant (pKa), molecular weight and protein binding are critical for placental transfer. Uncharged, low-molecular-weight (< 500 Da), lipid-soluble and unbound compounds can easily cross the human placenta (Garland, 1998; Syme *et al*., 2004). Anecdotal reports on fetal drug concentrations of chemotherapeutic agents reveal a wide variance in results (Germann *et al*., 2004; Mir *et al*., 2008a). Therefore, a systematic study is required in order to obtain clinically useful data on the most frequently applied chemotherapeutic drugs in pregnant patients.

First, transplacental transfer of cytotoxic agents was studied in a mouse model (Van Calsteren *et al*., 2010e). Ninety minutes after IV drug injection on gestational day 18.5, maternal and fetal blood samples were collected simultaneously. Plasma drug levels were determined using high-performance liquid chromatography (HPLC) or atomic absorption spectrometry (AAS). Fetal plasma concentrations of doxorubicin, epirubicin and daunorubicin were 5.1 ± 0.6%, 4.8 ± 3.8%, 13.3 ± 3.5% (mean ± SD) of the maternal concentrations, respectively. For vinblastine and cytarabine, fetal plasma concentrations were 13.8 ± 5.8% and 56.7 ± 22.6% of the maternal concentrations, respectively. Carboplatin fully passed the mouse placenta (117.0 ± 38.9%), whereas paclitaxel could not be detected in fetal plasma. These results show a high variability in fetal exposure between the different agents. However, except for carboplatin, all fetal drug levels were much lower than the respective maternal levels (Table II) (Van Calsteren *et al*., 2010e).

Although these results were reassuring, important limitations of these experiments must be taken into account: only one drug could be administered, whereas in clinical practice combination therapy is used; and the rodent placenta differs markedly from the human placenta. While placentation in humans is haemomonochorial, placentation in mice and rat

Table II. — Results of transplacental transfer of chemotherapeutic agents in a mouse and baboon model, based on simultaneously collected maternal and foetal plasma samples.

Drug	**Mouse (%)**	(Samples)	**Baboon (%)**	(Samples)
Doxorubicin	5.1 ± 0.6	(n = 8)	7.5 ± 3.2	(n = 6) (n = 9 < LLQ in foetus; n = 2 not simultaneously collected)
Epirubicin	4.8 ± 3.8	(n = 8)	4.0 ± 1.6	(n = 8) (n = 3 < LLQ in foetus).
Daunorubicin	13.3 ± 3.5	(n = 3)	Not tested	
Carboplatin	117.0 ± 38.9	(n = 6)	57.5 ± 14.2	(n = 7)
Cytarabine	56.7 ± 22.6	(n = 6)	Not tested	
Paclitaxel	Not detectable in fetus	(n = 6)	1.4 ± 0.8	(n = 7) (n = 4 < LLQ in foetus)
Docetaxel	Not tested		/	(n = 9 < LLQ in foetus)
4-OH-cylophosphamide	Not tested		25.1 ± 6.3	(n = 3) (n = 1 < LLQ in foetus and mother)
Vinblastine	13.8 ± 5.8	(n = 6)	18.5 ± 15.5	(n = 9) (n = 1 < LLQ).

LLQ, lower limit of quantification.

is labyrinthine with a three-layer tropoblast (Carter, 2007). In order to achieve results that could more readily extrapolated to the human setting, an animal model with a close phylogenetic relationship to humans was prioritised. Such model would need to demonstrate sufficient similarity with humans with respect to embryological development, placental structure and function, reproductive physiology and endocrinology, and drug metabolism. The baboon model was shown to address these requirements (Van Calsteren *et al*., 2009a). Subsequently, transplacental passage of chemotherapeutic agents was studied for the most frequently applied combination regimens in pregnant women (Van Calsteren *et al*., 2010b; Van Calsteren *et al*., 2010c): 5-fluorouracil-epirubicin-cyclophosphamide (FEC) – a standard treatment for breast cancer (Trudeau *et al*., 2005), doxorubicin-bleomycin-vinblastine-dacarbazine (ABVD) – the standard treatment for Hodgkin disease (Iannitto *et al*., 2009), and taxanes and carboplatin which are used in the treatment of cervical, breast and ovarian cancer (Amant *et al*., 2009).

The administered regimens were: FEC 100% (n = 2), FEC 200% (n = 1), ABVD 100% (n = 5), ABVD 200% (n = 1), docetaxel-trastuzumab (n = 1), paclitaxel (n = 2), docetaxel (n = 2), paclitaxel-carboplatin 100% (n = 2), paclitaxel-carboplatin 50% (n = 1), docetaxel-carboplatin (n = 1).

At predefined time-points over the first 76 h after the start of the drug infusion, fetal and maternal blood samples, amniotic fluid (AF) and maternal urine were collected simultaneously. Fetal and maternal tissues were collected during necropsy. HPLC, liquid-chromatography mass-spectrometry (LC-MS), enzyme-linked immunosorbent assay (ELISA) and AAS were used for bio-analysis of doxorubicin, epirubicin, vinblastine, cyclophosphamide and (4-hydroxy-) cyclophosphamide, trastuzumab, docetaxel, paclitaxel and total platin (Van Calsteren *et al*., 2010b; Van Calsteren *et al*., 2010c).

Transfer rate was calculated based on comparison of simultaneously collected samples in maternal and fetal plasma. The results are summarised in Table II.

For ***doxorubicin and epirubicin***, less than 10% of maternal concentrations was measured in the fetal compartment, in line with expectations based on molecular properties, i.e. a high molecular weight (527 g/mol) and protein binding (50-85%) (De Vita *et al*., 2001). Moreover, these drugs are substrates to ABC-transporters like P-gp, an efflux transporter for various xenobiotics which is expressed in bile canaliculi but also in the placenta (Mir *et al*., 2008b) and might explain the limited transplacental transfer of these anthracyclines. The transfer rate in the baboon was consistent in blood and tissues and paralleled passage rates in mice. The low transplacental transfer of doxorubicin and epirubicin is reassuring with regard to fetal toxicity and long-term effects. However, the unknown susceptibility of the fetal tissues to even low cytotoxic drug concentrations needs to be further explored. In particular, the fetal heart may be drug-sensitive since anthracyclines induce a dose-related cardiotoxicity (Lipshultz *et al*., 1991). To date, case series do not show impaired heart function or morphology in children after prenatal exposure to anthracycline-based chemotherapy (Meyer-Wittkopf *et al*., 2001; Aviles *et al*., 2006). The low levels of anthracyclines in the fetal heart may contribute to a favorable cardiac outcome in the offspring.

Vinblastine is highly protein bound (99%), has a high molecular weight (811g/mol) and is a substrate of ABC-transporters like P-gp and MRP. These drug properties contribute to a low transplacental transfer of 18.5 ± 15.5% (n = 9), as demonstrated in this baboon study.

Cyclophosphamide (CP) is an inactive prodrug converted by hepatic microsomal enzymes into reactive

intermediates, of which 4-OHCP is the most important. Plasma protein binding of CP is low (12-14%) resulting in easy penetration of most membranes (De Vita *et al*., 2001). Therefore, it was assumed that CP would readily pass the human placenta (Wiebe and Sipila, 1994). On the other hand, 4-OHCP is bound more tightly to plasma protein (50%), and the placental diffusion rate in mice was lower than for CP (Gibson and Becker, 1971). The results from our baboon study indeed showed a complete transplacental passage of CP (100%), but the 4-OH metabolite was detected in much lower concentrations in the fetus (25.1 ± 6.3% (n = 3)). This can be explained by the reduced or absent oxidative metabolism capacity of CP in the fetus, thereby limiting the conversion of the inactive parent compound into the active 4-OH metabolite. Moreover, maternal 4-OHCP binding to plasma proteins is stronger, thus reducing transplacental transfer. Although the fetal exposure to the active metabolite of CP appears to be limited, further research is warranted to investigate the possible late effects of CP on induction of secondary neoplasms (bladder cancer, leukaemias) and its potential detrimental impact on gonadal function in young adults.

We documented a ***carboplatin*** maternal-fetal transfer of 57.5 ± 14.2% (n = 7). Carboplatin is known to be bound to plasma proteins only for 24-50% (De Vita *et al*., 2001). The high free drug fraction and relatively low molecular weight (371 g/mol) explain the substantial tranplacental passage.

Comparison of fetal and maternal plasma levels of ***paclitaxel and docetaxel*** revealed a very low placental passage. The concentration of paclitaxel in fetal plasma was 1.4 ± 0.8% of maternal concentrations (n = 7). Docetaxel could not be detected in fetal plasma samples (n = 9). However, measurements in fetal and maternal tissues showed a delayed, but important distribution of taxanes into the fetal compartment.

Paclitaxel and docetaxel have a high molecular weight (854 and 862 g/mol), are highly lipid-soluble, exhibit a wide tissue distribution, are highly protein-bound (> 80-90%) and, consequently, have a long half-life. Furthermore, taxanes are substrates of ABC transporters like P-gp and MRP. These molecular characteristics of taxanes explain the fast distribution, the relatively low plasma levels and the slow elimination. The fetus appears to act as a 'deep' compartment, in which drugs are stored until maternal plasma concentrations decline. Taxanes are extensively metabolised in the liver by the cytochrome-P450-isoenzymes CYP2C8 and CYP3A4 (paclitaxel) and CYP3A4/5 (docetaxel). The maturation of these cytochromes occurs mainly in the first weeks of neonatal life (Mir *et al*., 2008b). Therefore, it can be assumed that fetuses are unable to metabolise taxanes and remain susceptible to their cytotoxic effects. The implication of the storage of taxanes in fetal tissues is unclear as yet.

Trastuzumab is a humanised monoclonal antibody targeting HER2-positive breast cancer cells. The major side-effect of trastuzumab is cardiotoxicity. Trastuzumab is also associated with oligohydramnios when administered during pregnancy. In the baboon, 85% and 3% of the maternal plasma concentration was detected in fetal plasma 2 h and 26 h, respectively, after trastuzumab infusion. Concentrations in fetal tissues, including the heart, varied between 5% and 14% of the maternal concentrations. In conclusion, a significant fraction of trastuzumab crosses the baboon placenta. The lack of mature reabsorption in the fetal kidney results in relatively high concentrations of trastuzumab in amniotic fluid. Trastuzumab-induced oligohydramnios might be related to HER2 inhibition in fetal nephrogenic cells and decreased fetal renal blood flow by VEGF inhibition. These results add to the knowledge that trastuzumab is associated with oligohydramnios. Fetal cardiotoxicity after *in utero* exposure to trastuzumab deserves further attention. Children exposed *in utero* to trastuzumab are candidates for nephrologic and cardiologic follow-up.

2B. Pharmacokinetics of chemotherapy in pregnancy (Van Calsteren et al., 2010d)

Apart from the potential toxicity of chemotherapeutics on the fetus, maternal effects were also studied. Most anticancer drugs exhibit a narrow therapeutic window with small margins between therapeutic toxic and effects. Interindividual pharmacokinetic and pharmacodynamic variabilities are generally substantial and may be augmented by pregnancy (Undevia *et al*., 2005). Indeed, physiological adaptations occur, including an expansion of plasma and extracellular fluid volume, alterations in hepatic function, increased glomerular filtration rate, changes in plasma protein concentrations and protein binding. These alterations will influence major pharmacokinetic processes such as absorption, distribution, metabolism and elimination and likely interfere with drug efficacy and toxicity (Krauer *et al*., 1980). Although important for all drugs, chemotherapeutic agents deserve our special attention given their strong actions and possible mutagenic and teratogenic effects.

The pharmacodynamic (antitumour activity and toxicity in relation to the dose administered) consequences of the above-mentioned physiological

changes for chemotherapeutic agents are difficult to predict without pharmacokinetic data. Therefore, it remains unknown whether the pregnant patient is treated optimally with standard chemotherapy regimens as applied in current clinical practice (Wiebe and Sipila, 1994; Cardonick and Iacobucci, 2004). To answer this crucial question, we initiated a study on the pharmacokinetic behaviour of chemotherapeutic agents, comparing the pharmacokinetic parameters in the pregnant and nonpregnant state, in both a clinical study and a baboon study.

To this end, standard-dosed chemotherapy regimens were administered in pregnant and nonpregnant baboons/women, followed by serial blood samplings. Drug plasma levels were determined using HPLC and AAS. A non-compartmental pharmacokinetic analysis with the determination of C_{max}, AUC, $t_{1/2}$, clearance and distribution volume was performed using WinNonLin Software (Van Calsteren *et al*., 2010d).

In a pregnant baboon model, we assessed the pharmacokinetic characteristics of doxorubicin (n = 3), paclitaxel (n = 2) and platinum (n = 2) during and after pregnancy. For the three drugs, a decrease in plasma drug exposure (AUC-D and C_{max}-D) and an increase in clearance and distribution volume was observed during gestation.

In the clinical trial, we performed a pooled analysis on 16 cycles from pregnant patients and 11 from nonpregnant patients. Numbers of pregnant/nonpregnant patients were 5/2, 7/5, 4/4 and 2/2 for paclitaxel, doxorubicin, epirubicin and platinum, respectively. For all drugs tested, a decreased area under the curve and maximal plasma concentration as well as an increased distribution volume and clearance were documented during pregnancy (Table III).

The results of the preclinical and clinical study are consistent, confirming the hypothesis that gestational physiological changes alter the pharmacokinetic patterns of cytotoxic drugs. Plasma drug exposure (AUC-D and C_{max}-D) is lower during pregnancy owing to an increase in clearance and distribution volume. Whether these changes are related to alterations in tissue/tumor drug exposure and drug efficacy, is not known at this stage and needs further research. However, these data clearly indicate that follow-up of mothers who received chemotherapy during pregnancy is warranted.

3. Effects of prenatal exposure to chemotherapy on the neurological outcome

During the second and third trimester of pregnancy, administration of chemotherapy is considered relatively safe in the short term (Cardonick and Iacobucci, 2004; Van Calsteren *et al*., 2010a). However, reliable data on the long-term outcome of children after prenatal exposure to chemotherapy are lacking. Fetal organogenesis ends around 8 weeks after conception for most organs, except for the brain and the gonads. Consequently, questions arise on the possible long-term effects of prenatal exposure to chemotherapeutic agents on the neurological development, fertility and carcinogenesis (Cardonick and Iacobucci, 2004; Zemlickis *et al*., 1992; Pentheroudakis and Pavlidis, 2006).

To date, available data on long-term follow-up of the children are poor. Only two series have been reported with a follow-up until school age. Unfortunately, the methodology was described poorly and/or the results may have been biased by parental opinion (Aviles and Neri, 2001; Hahn *et al*., 2006). Aviles *et al*. described a series of 84 children from mothers with haematological malignancies who received chemotherapy during pregnancy. The children were examined for physical health, growth, general development and haematological, cytogenetic, neurological, psychological and learning disorders. However, no details on the neurological and psychological tests were provided. They reported that all children, including 12 second-generation children, had a normal birth weight, a normal learning and educational performance, and no congenital, neurological or psychological abnormalities. With a median follow-up of 18.7 years (range 6-29 years), no malignancies had been observed (Aviles and Neri, 2001). Nevertheless, it is somewhat surprising not to see any problems in a group of 96 children.

Hahn *et al*. reported on the outcome of 40 children (age 2-157 months) after *in utero* exposure to chemotherapy for breast cancer. The parents and teachers of the children were asked to participate in a telephone/mail survey on the development and health of the children. In this series, 2 out of 18 school-going children were reported to have special educational needs. In 43% of children, no medical problems were reported. The problems registered in the remaining cases included allergy, eczema, asthma and upper-airway infections (Hahn *et al*., 2006).

3A. Study of brain development and behaviour of the offspring after vinblastine and doxorubicin administration to pregnant mice (Van Calsteren et al., 2009b)

Searching for the target areas of potential neurological damage in children prenatally exposed to chemotherapy, we performed a preclinical study in a mouse model with both morphological and functional examination of the offspring. Different

Table III. — Pooled analysis of pharmacokinetic parameters of paclitaxel, carboplatin, doxorubicin and epirubicin in pregnant and control patients.

	Paclitaxel		**Carboplatin**		**Doxorubicin**		**Epirubicin**	
	Pregnant (n = 5)	Control (n = 2)	Pregnant (n = 2)	Control (n = 2)	Pregnant (n = 7)	Control (n = 5)	Pregnant (n = 4)	Control (n = 4)
Age (year)	32.0 ± 4.7	31.0 ± 4.2	31.0 ± 4.2	31.0 ± 4.2	32.0 ± 2.8	33.4 ± 13.2	36.3 ± 2.9	36.3 ± 8.1
GA (weeks)	26.2 ± 3.6	-	24.0 ± 1.4	-	29.0 ± 3.8	-	28.0 ± 6.1	-
BSA (m^2)	1.8 ± 0.1	1.8 ± 0.1	1.9 ± 0.2	1.8 ± 0.1	1.9 ± 0.2	1.8 ± 0.2	2.0 ± 0.2	1.6 ± 0.1
Vd (l)	862.1 ± 518.9	513.4 ± 34.2	378.8 ± 76.4	272.7 ± 1.3	2486.3 ± 656.8	1915.3 ± 317.6	2710.4 ± 325.6	2236.0 ± 493.1
$t_{1/2}$ (h)	16.7 ± 8.4	12.5 ± 2.0	23.7 ± 8.6	28.9 ± 9.9	25.6 ± 7.7	25.5 ± 5.6	19.4 ± 3.4	22.8 ± 5.9
Clearance (l/h)	34.7 ± 4.3	28.7 ± 2.7	11.4 ± 1.9	6.9 ± 2.3	68.6 ± 9.4	54.1 ± 13.7	98.0 ± 11.6*	68.7 ± 7.4*
Cmax-D*IT (ng/ml/mg*h)	21.8 ± 7.1	39.8 ± 2.3	7.6 ± 4.3	11.9 ± 1.7	5.9 ± 2.4*	8.9 ± 1.2*	5.1 ± 1.4*	8.5 ± 1.2*
AUC-D (h*ng/ml/mg)	29.2 ± 3.7	35.1 ± 3.3	88.9 ± 14.9	152.7 ± 51.4	14.9 ± 2.3	19.7 ± 6.3	10.3 ± 1.2*	14.2 ± 0.8*

* significant difference between pregnant and nonpregnant patients:$p < 0.05$ (Wilcoxon Rank Sum test).
GA, gestational age; BSA, body surface area; Vd, distribution volume; $t_{1/2}$, terminal half life; C_{max}-D*IT, maximal plasma concentration corrected for dose and infusion time; AUC-D, area under the curve corrected for dose.

dosages of doxorubicin, vinblastine or saline were administered to pregnant C57BL/6J mouse dams on gestational day 17.5. Both immediate (24 h post-injection) and residual (4-5 months post-injection) brain damage were investigated by light- and electron microscopy, and a battery of behavioural tests was performed in 3 months-old offspring.

Exposure of high dosages of doxorubicin and vinblastine caused transient regionally limited lesions to the brain microvasculature and surrounding parenchyma, and an inconsistent development of cortical ectopias resembling microgyri and isolated lissencephaly type 2-like overmigrations. These lesions could not be detected in 3 months-old mice, suggesting absence of permanent morphologic damage.

Functional testing of drug-exposed mice offspring indicated a few subtle, but specific alterations in their neurobehavioural profile. No learning impairment was consistently observed, but several tests indicated changes in the emotional behaviour and increased anxiety in the drug-exposed mice.

In conclusion, a thorough morphological neurological assessment revealed subtle changes in the brain. This conclusion underscores the need for long-term follow-up with a special emphasis on the neurological outcome.

3B. Clinical study

In the absence of a standardized follow-up of children exposed *in utero* to chemotherapy, we initiated a prospective multicentric international study (University Hospital Gasthuisberg Leuven, St Radboud Hospital Nijmegen and Motol University Hospital Prague). The strength of this study lies in its robustness: all children are clinically examined with subsequent determination of the Bayley score at 18 months, and a battery of neuropsychological tests focusing on working memory and attention at the ages of 5-6 years, 8-9 years, 11-12 years and 14-15 years. A preliminary analysis performed for my PhD thesis showed an age- adequate development in most children, yet a larger population and a longer follow-up period will be necessary to be able to confirm or refute the preliminary results.

Conclusion

The results obtained in this study suggest that *in utero* exposure to chemotherapy does not deteriorate neonatal outcome. This finding is explained by the fact that chemotherapy is not administered during the most vulnerable period of gestation, namely the first trimester. Also the placental barrier function protects the fetus from the toxic effects of chemotherapy and contributes to the reassuring findings obtained in the children. However, before definitive conclusions can be drawn, more children should be recruited and a longer follow-up period is required.

Plasma chemotherapy exposure is lower during pregnancy than in nonpregnant women. Further research is necessary to investigate whether these alterations include a lower tumour toxicity of chemotherapeutic agents, which is necessary to evaluate whether pregnant women receive optimal chemotherapy treatment at this time. Therefore, this research project is continued and expanded nationally and internationally.

Acknowledgements

We are very grateful to Liesbeth Heyns, Rieta Van Bree, Rene Verbesselt, Jan de Hoon, Roland Devlieger, Luc De Catte, Ernst de Bruijn, Daniel Chai and Sonia Demarsin for their assistance in this research.

This research is supported by Research Foundation-Flanders (F.W.O.) Project G. 0358.06, 'Stichting tegen kanker Project SCIE2006-17', Research Fund-K.U.Leuven (OT/07/053), Clinical Research Fund-UZ Gasthuisberg and FOD NKP 29 038. Frédéric Amant is clinical researcher for Research Foundation-Flanders (F.W.O.); Kristel Van Calsteren is aspirant for Research Foundation-Flanders (F.W.O.).

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