Antihypertensive Treatment of Pre-eclampsia;

Pharmacological aspects of ketanserin and nicardipine

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ABSTRACT

(Very) High blood pressure in pre-eclamptic women poses a serious risk of acute cerebrovascular complications in these women, necessitating the use of antihypertensive treatment in pre-eclampsia. In this review the different antihypertensive drugs, used in the management of pre-eclampsia, are discussed with a focus on efficacy and maternal and neonatal safety. The characteristics of each drug are summarized, including placental transfer, and comparisons between antihypertensive drugs are shown, based on recent publications. Special emphasis is given on newer developments including treatment with ketanserin or nicardipine.

INTRODUCTION

Pre-eclampsia is a multisystem disorder with an incidence in pregnancy varying between 2% and 7% [1] and it is still the leading cause of maternal mortality during pregnancy in the Netherlands [2].

The disorder is characterised by the occurrence after the 20th week of gestation, of elevated maternal blood pressure and proteinuria. In most women, the onset is near term or intrapartum and the disease is usually mild with a negligible increased risk of adverse pregnancy outcome. However, in severe cases, pre-eclampsia can lead to serious maternal complications, such as HELLP-syndrome (haemolysis, elevated liver enzymes, low platelet counts), pulmonary edema, acute renal failure, liver failure or haemorrhage, abruptio placentae and eclampsia. The major cause of maternal death is cerebral haemorrhage.

Pre-eclampsia is also associated with increased perinatal morbidity and mortality. Depending on the severity of the disease, intrauterine growth restriction, reduced amniotic fluid and abnormal oxygenation can occur, leading to increased occurrence of iatrogenic preterm delivery, small-for-gestational-age (SGA) neonates and prenatal or perinatal death [3].

PATHOGENESIS

The cause(s) of pre-eclampsia still remain unknown, but most hypothesis focus on vascular dysfunction and maternal- foetal (paternal) immune maladaptation [3].

The presence of the placenta or the maternal response to placentation is considered to be a key-factor in the disorder. Cytotrophoblastic invasion of the spiral arteries is incomplete in pre-eclampsia, resulting in narrow and undilated myometrial segments [4], which may compromise uterine blood flow during pregnancy. Recently, the anti-angiogenic protein sFlt-1 (soluble fms-like tyrosine kinase or sVEGFR-1) has been shown to be elevated in pre-eclampsia as compared to normal pregnancies. This protein is thought to be involved in antagonising VEGF (vascular endothelial growth factor) and PlGF (placental growth factor), which may cause the endothelial dysfunction, known to be present in pre-eclampsia [4,5]. In the future, this mechanism may become a possibility for an alternative and more causal strategy for pharmacological management of pre-eclampsia.

An important factor, implicated in the pathogenesis of pre-eclampsia is the close tissue contact between maternal and foetal cells, resulting from the trophoblast-cell invasion into the deciduas [3]. Couple-specific immune maladaption is speculated to be involved in the superficial placentation, causing increased apoptosis of cytotrophoblasts. This may trigger a systemic inflammatory response in the mother, resulting in endothelial activation and inflammation and thrombocyte activation. The latter can result in a lower production of the

vasodilator prostacyclin and increased release of the vasoconstrictors thromboxane A2 and serotonin.

PREVENTION

One of the main problems of pre-eclampsia is that the clinical symptoms become manifest long after the compromised placentation and vascular dysfunction have developed. Management at the time of diagnosis is limited to controlling maternal symptoms, and assuring foetal well-being. Theoretically, pharmacological interventions at the time of early placentation and before the occurrence of extensive vascular dysfunction, would be needed to prevent the development of pre-eclampsia or reduce the severity of the disease. However, early identification of women at risk for developing pre-eclampsia remains an unresolved challenge in obstetric practice, hampering the possibility of selective use of prophylactic drugs for high-risk women.

Acetylsalicyl acid is the drug most widely used to prevent pre-eclampsia, based on its positive effect on the imbalance in the tromboxane-A2 to prostacyclin ratio, found in pre-eclampsia. A review [6] of 32 trials (n=29331) with acetylsalicyl acid showed a 15% reduction in the occurrence of pre-eclampsia (95% CI 0.78-0.92). Dosages of acetylsalicyl acid and time of intake have been demonstrated to influence efficacy, with daily dosages ≥ 75mg and intake before bed time showing the highest effect. However, a recent placebo-controlled study in 3294 nulliparous women did not show an effect on incidence of pre-eclampsia or gestational hypertension of 100 mg acetylsalicyl acid from 14-20 week's gestation [7]. To date, it still remains unclear which women are most such likely to benefit from acetylsalicyl acid, at what dosage and at what gestational age treatment should be started

Based on the hypothesis that antioxidants may be effective in decreasing oxidative stress and improving vascular endothelial function, 283 high risk women were supplemented in a placebo controlled study with vitamin C (1000 mg) and vitamin E (400 IE) from the 16th-22th week gestation [8]. The occurrence of pre-eclampsia was reduced from 17% to 8% in the treated group ($p < 0.02$), but no reduction on the occurrence of severe pre-eclampsia or improvement in perinatal outcome could be established. Currently, a large trial is undertaken in the UK, to assess the influence of vitamin C and vitamin E on pre-eclampsia.

The influence of nutritional interventions such as zinc, magnesium or fish oil supplementation has been reviewed but no differences in incidence of hypertension during pregnancy or pre-eclampsia could be demonstrated [9]. Calcium supplementation has been associated with a five-fold reduced risk on hypertension and pre-eclampsia, but only in patients with low baseline dietary calcium intake (RR 0.22; 95% CI 0.16-0.49) [10]. In developed countries, its use is therefore not recommended.

Antihypertensive drugs (alfa-agonists, calcium-channel blockers, beta-adrenoceptor blockers or hydralazin) have been used orally in women with chronic hypertension with a beneficial effect on the occurrence of severe hypertension, but use of these drugs did not decrease the risk of pre-eclampsia according to a Cochrane review, referencing 40 clinical trials with 3797 women [11]. The possibility of the occurrence of adverse effects, such as reduced birth weight in patients treated with beta-adrenoreceptor blockers [12], should be taken into account in the decision to treat prophylactic ally with oral antihypertensive drugs.

Following the increased incidence of thrombophilia and hyperhomocysteinemia, demonstrated in women with a history of pre-eclampsia [13], administration of heparin or low-molecular heparin has been found to reduce the incidence of pre-eclampsia in women with thrombophilia [14]. However, due to the potential risks of anticoagulant treatment during pregnancy, its use is currently only recommend in a trial-setting.

In summary, there is no efficient prophylactic treatment available to prevent pre-eclampsia.

MAGNESIUM SULPHATE

The use of magnesium sulphate in pre-eclamptic patients is based on the efficacy of the drug in preventing eclampsia in women with severe pre-eclampsia. As shown in a systematic review [15] and in a large randomised controlled trial (the Magpie trial [16]) comparing magnesium sulphate with placebo in 10141 women, magnesium sulphate was associated with a significantly reduced rate of eclampsia (RR 0.41; 95% CI 0.29-0.58). In mild pre-eclampsia, no impact on disease progression was found [17].

Current dosage schedule consist of a bolus infusion of 4 to 6 g magnesium sulphate, followed by infusion of 1 g/h during 24 h. Because of its vasodilatating effect, magnesium sulphate may lower maternal blood pressure, albeit not extensively. The drug can cause respiratory depression, nausea, drowsiness, confusion and flushing as side-effects in the mother.

ANTIHYPERTENSIVE MANAGEMENT OF PRE-ECLAMPSIA

Delivery is the only cure for pre-eclampsia. In pre-eclampsia occurring in near-term or term patients, delivery is, therefore, recommended to minimize the risk of maternal complications. The use of antihypertensive drugs may be necessary to stabilise maternal blood pressure before delivery and to gain sufficient time to administer corticosteroids in patients with a gestational age below 34 weeks to enhance foetal-lung maturity [18].

In severe pre-eclampsia occurring before 32 weeks of gestation, expectant management by postponing delivery using antihypertensive drugs in patients with a stable maternal and foetal condition, can be considered to improve neonatal outcome [19,20]. A recent review [21] showed that a mean pregnancy prolongation in early pre-eclampsia using antihypertensive treatment of 10-14 days can be established, which is considered clinically important for an improvement in neonatal outcome. Some obstetricians, however, prefer early delivery after stabilizing the maternal condition (interventionist care) to prevent the development of serious maternal complications. A meta-analysis on available studies on interventionist versus expectant care [22] stated that due to lack of randomised trials of sufficient size, no conclusions can be drawn. Recent trials show that short-term morbidity for the baby may be reduced by a policy of expectant care [23,24]. However, improvement in perinatal outcome should never be achieved at the expense of maternal safety. This implies that only in tertiary care settings with experienced personnel, expectant management using adequate monitoring of both mother and foetus, should be considered in severe early-onset pre-eclamptic patients.

The challenge in using antihypertensive drugs in pre-eclampsia is to reduce blood pressure to assure maternal safety, while at the same time not compromising uteroplacental perfusion.

There is a general consensus [25] to start antihypertensive treatment in a pregnant women with sustained values of systolic blood pressures of 170 mmHg or above and diastolic blood pressures of 110 mmHg or above. The aim of such treatment is to lower the risk of harmful effects, especially stroke and other haemorrhagic complications, for the mother. Whether to target at a fixed value of systolic and diastolic blood pressure or to aim for a reduction relative to the initial maternal blood pressure remains controversial. In patients with an initial high blood pressure, the latter may lower the risk of acute foetal distress, caused by diminished uteroplacental perfusion following aggressive antihypertensive treatment, but in patients with borderline blood pressure, a relative reduction might result in over-treatment.

The ideal antihypertensive drug for treatment of severe hypertension in pregnancy should be potent, rapidly acting, controllable and without detrimental maternal or foetal side effects. Unfortunately, only limited antihypertensive drugs are studied and even less are licensed for use in pre-eclamptic patients. As a result, most drugs currently used in pre-eclampsia, have long been surpassed by newer drugs in other medical areas.

Administering antihypertensive drugs in pre-eclampsia is only treating the symptoms of the disease. As long as the foetus and placenta is present, the disease will not be cured and may even exacerbate during treatment. To maintain adequate blood pressure control, increased dosages, a switch from oral to parenteral administration or combination treatment are often necessary, but no consensus on choice of first-, second- or even third-line treatment is available. Considering the fact that current antihypertensive drugs differ greatly in their action, potency and side-effects, it is surprising that (inter)national recommendations on which drug to prefer, are still lacking. In the next part, the different drugs are discussed

with their respective properties (Table 1.1), which may be helpful for developing treatment protocols.

Table 1.1 Antihypertensive drugs for management of pre-eclampsia

Drug	Mode of action	Route of administration	Dosage	Onset of action	Duration of action
Methyldopa	Alfa-2-receptor agonist	Oral	500-1,000 mg tid or qid	4-6 h	20-24 h
Nifedipine	Calcium-channel blocking agent	Oral retard Oral oros Short-acting capsule	10-40 mg bid 30-90 mg qd 5-10 mg every 2-6 h	0.5-1 h 2-4 h 10-15 min	6-11 h 24 h 4-5 h
Nicardipine	Calcium -channel blocking agent	Oral[1] Intravenous (central line)	20 mg tid 3 mg/h bolus, followed by 0.5-10 mg/h	20 min 5-10 min	1-2 h
Dihydralazin	Arterial vasodilator	Intravenous	Bolus 5 –10 mg in 30-60 min, followed by 1-10 mg/h	10-20 min	3-8 h
Hydralazin	Arterial vasodilator	Oral Intravenous	25-50 mg tid or qid 5–10 mg iv every 20-30 minutes	1 h 10-20 min	3-8 h
Labetalol	Alpha-1 and beta receptor antagonist	Oral Intravenous	50-200 mg tid 10-30 mg/h[2]	1-4 h 5-10 min	4-6 h
Ketanserin	5-HT_{2A}-receptor antagonist	Oral Intravenous	20-40 mg bid 5-10 mg bolus, followed by 2-14 mg/h	0.5-2 h 1-3 min	13-18 h

[1] limited data

[2] higher dosages have been used

Methyldopa (Aldomet®)

Methyldopa is an oral drug with an excellent safety record for use in pregnant women [26-28]. The drug exert its antihypertensive effect from the action of its metabolite α- methylnorepinefrine on central inhibitory α-adrenoreceptors. Common dosages of methyldopa are 500-1,000 mg orally taken 3-4 times daily, with a maximum of 4 g/day.

In mild pre-eclampsia and in the management of chronic hypertension in pregnant women, methyldopa is regarded as drug of choice, but its limited efficacy and delayed onset of action (4-6 h) preclude its use in acute, severe pre-eclampsia. Side-effects include decreased heart rate and sedation.

Hydralazin (Apresolin®) or dihydralazin (Nepresol®)

Hydralazin and dihydralazin differ slightly in their molecular structure, but both act in a similar way as vasodilatating agents on arterial smooth muscle, causing a reduction in total peripheral vascular resistance and reflex tachycardia. The antihypertensive effects occur 10-20 min after intravenous administration, following metabolism in the vessel wall. The duration of action is 3-8 h [29]. The drugs are very effective in lowering maternal blood pressure, but maternal side-effects (headache, nausea, epigastric pain and fluid retention) are common and mimic symptoms of deteriorating pre-eclampsia. Careful monitoring of central venous pressure and adequate volume expansion is necessary in patients receiving (di)hydralazin, to prevent an excessive hypotensive effect, causing foetal distress [30].

Dosage schedules vary greatly, with repeated bolus administrations of hydralazin preferred in the US to prevent hypotensive overshoots [31,32]. In the Netherlands, dihydralazin is commonly used as a continuous intravenous infusion starting with 1 mg/h after a bolus infusion in 30-60 min of 5-10 mg, to a maximum of 10 mg/h. The availability of intravenous dihydralazine has been severly limited in the past years. Intravenous hydralazin is still generically available in the US and some European countries.

Intravenous (di)hydralazin has been regarded as the drug of choice in pre-eclamptic patients for many years [25]. Recently, however, a meta-analysis [33] of 21 available randomised clinical trials for the treatment of severe hypertension (including 1,085 women) showed that hydralazin was associated with poorer maternal and perinatal outcomes than other antihypertensive drugs, particularly nifedipine and labetalol. Hydralazin was associated with a trend toward more persistent severe hypertension compared to nifedipine or isradipine [29% (0%-32%) vs 19% (0%-40%)], RR 1.41 (0.95-2.1) and with the use of additional antihypertensive drugs [13% (0%-32%) for hydralazin vs 5% (0-24%) for nifedipine, RR 2.13 (1.2-3.9)]. Seemingly contradictory, hydralazin was also found to be associated with more maternal hypotension than other antihypertensive drugs (labetalol, nifedipine, isradipine, ketanserin or uradipil) [0% (0%-67%) vs 0% (0%-17%), RR 3.29 (1.5-7.23)]. Other maternal complications occurred more often with hydralazin than with other antihypertensive drugs; caesarean section [67% (8%-100%) vs 59% (5%-100%)]; placental abruption [18% (3%-20%) vs 0% (0%-2%)] and maternal oliguria [17% (4%-41%) vs 0% (0%-9%)]. Hydralazin was associated with more adverse effects on foetal heart rate than other antihypertensive drugs [11% (0%-56%) vs 0% (0%-50%)]. Hydralazin was also associated with more low APGAR scores at 1 min [67% (38%-83%) vs. 15% (14%- 67%)] but not at 5 min, and a trend towards an increase in stillbirth [0% (0%- 31%) vs 0% (0%- 22%), risk difference 0.02 (-0.01 to 0.05)].

However, the number of events ranged widely within trials, numbers of participants in most trials were small and a large heterogeneity between trials was present (e.g. mixed populations of either pre-existing hypertension or gestational hypertension with or without proteinuria were enrolled). Importantly, dosages of hydralazin and schedules of administration varied

widely between trials. Probably the main conclusion that can be drawn, is that (di)hydralazin is a drug that should be used only in experienced hands in a well-designed dosage schedule, and in a setting in which careful monitoring of maternal and foetal conditions is available.

Labetalol (Trandate®)

Labetalol is a selective alpha-blocking and non-selective beta-blocking agent, lowering high blood pressure while, at the same time, exerting a heart-rate-reducing effect because of the drug's beta-blocking effect. The drug is used in dosages of 10 to 30 mg/h intravenously or 50 to 200 mg orally three times daily. After intravenous administration, the antihypertensive effect occurs immediately with a duration of action of 4-6 h, whereas, after oral administration the effect is delayed for 1-4 h. The drug is tolerated fairly well, with flushing, nausea and vomiting as main side effects. Due to the beta-blocking effect, its use is contra-indicated in patients with congestive heart failure, asthma or bradycardia [29].

Oral labetalol has been used extensively in mild to moderate hypertension in pregnancy and pre-eclampsia [28,34]. The drug has shown to be more effective than methyldopa in decreasing blood pressure [35].

In severe pre-eclampsia, high intravenous dosages of labetalol (up to 2,400 mg/day) are sometimes necessary to obtain adequate maternal blood pressure in pre-eclamptic patients. However, these high maternal dosages of labetalol have been associated with the occurrence of severe neonatal bradycardia, hypotension and hypoglycaemia [36-38]. These neonatal side-effects are the major limitation for its use in severe pre-eclampsia.

Ketanserin (Ketensin®)

A drug with a different action is ketanserin. Ketanserin is a selective 5-HT_{2A} receptor-blocking agent with minor alpha-1-receptor-blocking properties. Its use in pre-eclampsia is based upon the assumption that serotonin is involved in the vasoconstrictive processes, leading to hypertension in pre-eclampsia. 5-HT_1 receptors are located mainly in the vascular endothelium, whereas 5-HT_2 receptors are localised in platelets and vascular smooth muscle cells. In pre-eclampsia, characterised by endothelial dysfunction and concomitant loss of 5-HT_1 endothelial receptors, free serotonin will stimulate mainly 5-HT_2 receptors in platelets causing platelet aggregation and serotonin release, and 5-HT_2 receptors in vascular smooth muscle, causing vasoconstriction. The observation that levels of platelet-derived free serotonin are found to be significantly higher in pre-eclamptic patients than in normal pregnancies [39] supports this theory.

Ketanserin acts as an antagonist on the 5-HT_{2A} receptor, counteracting the serotonin-depending vasoconstriction and platelet aggregation. The latter may be clinically advantageous, especially in pregnancies complicated with HELLP-syndrome [40].

The pharmacokinetic characteristics of the drug are not ideal for drug treatment in pre-eclampsia because of its long elimination half-life of 10-18.5 h, which precludes accurate

titration and may lead to accumulation. Ketanserin is oxidized in the liver via mixed-function oxidases to inactive 6-hydroxy ketanserin or reduced to ketanserinol. The latter metabolite is considered inactive but may be re-oxidized to form ketanserin again [41].

Side effects of ketanserin are mainly dizziness, dryness of the mouth, nasal congestion and tiredness. A potentially serious adverse effect of ketanserin is prolongation of the QT_c interval [42] and treatment should be accompanied by ECG control.

The drug is licensed for use in pre-eclampsia but its availability is limited to some European countries and South Africa. In the Netherlands the drug is a first choice drug in settings in which invasive monitoring of mother and foetus is not feasible, based upon its ease in handling.

Conflicting data exist on the efficacy of ketanserin. Bolte et al [43] compared intravenous ketanserin (5 mg bolus, followed by a continuous infusion of 4 mg/h to a maximum of 10 mg/h) with intravenous dihydralazin (no bolus, continuous infusion of 1 mg/h to a maximum of 10 mg/h) in 31 patients with severe early-onset pre-eclampsia and found both drugs to achieve adequate blood pressure control. Ketanserin acted more rapidly, but showed, at the same time, a more gradual decline in blood pressure without the occurrence of reflex tachycardia. In another trial, with 44 early-onset pre-eclamptic patients using a similar dosage schedule, Bolte et al [44] also showed that ketanserin resulted in a better blood pressure control and less maternal side effects than dihydralazin. However, the dosage-schedule of dihydralazin did not comprise a bolus infusion as starting dosage, which makes comparisons of time to target blood pressure hazardous. Also, significantly more patients treated with ketanserin needed antihypertensive co-medication to maintain adequate blood pressure control. A Cochrane review [45] on three available studies of ketanserin versus dihydralazin found that use of ketanserin was associated with persistent high blood pressure as compared to dihydralazin (28/74 vs 4/70, RR 6.74 [2.49-18.28]).

These data make the role of ketanserin in the treatment of pre-eclampsia ambiguous.

Calcium-channel blocking agents

Calcium-channel blocking agents inhibit the influx of calcium via voltage dependent, slow L-type calcium channels, causing peripheral vasodilatation. The safety of the use of calcium-channel blocking agents of the dihydropyridine type in pregnant women has long been debated. In animal studies [46,47], the use of nifedipine and nicardipine has been associated with foetal acidemic responses, caused by a decrease of uteroplacental perfusion. However, in women with pregnancy-induced hypertension, nifedipine was not associated with a negative effect on uteroplacental blood flow [48], and the same conclusion was drawn after reviewing the extensive experience with nifedipine as a tocolytical drug in recent years [49]. Probably, the adverse foetal effects found in animals are linked to the substantially elevated

dosages as compared to dosages used as tocolytical or antihypertensive drug in pregnant women.

The successful use of nifedipine in pre-eclampsia has been described in several studies [50, 51] and, as cited above, a recent review showed a favourable outcome of nifedipine as compared to parenteral hydralazin [33]. Current dosage schedules of nifedipine are 20-90 mg orally daily as a sustained release formulation. In treating hypertension in non-pregnant patients, the short-acting nifedipine has been associated with excess cardiovascular morbidity and mortality and its use has been replaced by sustained release formulations, both in non-pregnant and pregnant patients [52]. However, these formulations are less effective and less controllable in acute situations, and nifedipine, therefore, may not be first choice treatment in severe pre-eclampsia [53].

Another concern in using nifedipine in pre-eclampsia has been the supposed risk of neuromuscular blockade during concomitant use of magnesium sulphate, as reported in case reports [54,55]. However, a recent study analysing 162 patients and 215 controls, showed that patients who received nifedipine and magnesium sulphate contemporaneously had no excess of neuromuscular weakness and no neuromuscular blockade (53%) vs control subjects who received antihypertensive drugs only (53.1%) or vs control subjects who receive no antihypertensive medication at all (44.8%) [56].

The advantages of the use of nifedipine are its ease of administration and its low costs. Side effects of nifedipine are limited and are mainly flushing, nausea and vomiting. The tocolytical effects of nifedipine might be disadvantageous in pre-eclamptic patients, delaying induction of labor or increasing the risk of postpartum haemorrhage [57]. Calcium-channel blocking agents have been associated with the occurrence of pulmonary oedema, possibly caused or enhanced by the cardiovascular action of the dihydropyridine derivates [58]. However, irrespective of antihypertensive treatment, pulmonary oedema is known to occur as a complication in pre-eclampsia, usually after iatrogenic fluid overload in combination with the administration of steroids for foetal lung maturation [29].

The lack of an immediate acting and well-controllable formulation of nifedipine and its cardio-depressant side-effects, stimulated the search for alternative calcium-channel blocking agents. The use of isradipin in pre-eclampsia has been found effective in small studies [59], but an intravenous formulation is not available in most countries.

Nicardipine (Cardene®) is a calcium-channel blocking agent, with some potential advantages over nifedipine in pre-eclampsia [60]. It acts more selectively on the vessels and causes less negative inotropic effects and reflex tachycardia. Another advantage is that the drug is available both for oral and intravenous administration, but intravenous administration should be performed through a central line because of the risk on phlebitis. The drug has a

short half-life of 2-5 min which increases after prolonged infusion to 1-2 h [61]. It is metabolised extensively in the liver to inactive metabolites.

Studies on its use in pre-eclamptic patients are limited. Carbonne [62] was the first to describe its use, intravenously, in 20 pre-eclamptic patients with a gestational age between 27 and 40 weeks with failure on oral antihypertensive treatment. Nicardipine was administered in a dosage of 2 to 6 mg/h, depending on body weight. Efficacy was evaluated by the time required to decrease diastolic BP below 90 mmHg. Target BP was reached in all patients within 70 – 120 min and treatment was sustained for 5 days (2-15 days). Doppler velocimetry remained stable and neonatal outcome showed no deleterious effects of the treatment. Maternal headache and increase in heart rate were the most common side effects.

The same group [63] compared oral nicardipine (3 dd 20 mg) to oral metoprolol (1 dd 200 mg slow-release) in 100 pregnant patients with moderate to mild hypertension (consisting of a mixed population of chronic hypertension, gestational hypertension and pre-eclampsia), starting after the 20th week of pregnancy. Nicardipine was more effective than metoprolol in reducing both systolic and diastolic BP. Treatment failure tended to be less frequent (n = 7 vs n = 15, $p < 0.06$) and at a later gestational age in the nicardipine group as compared to the metoprolol group (35 ± 2.6 weeks vs 31.4 ± 4.4 weeks). The umbilical artery resistance was lower as well as the incidence of caesarean delivery for foetal distress in the nicardipine group. No significant difference in neonatal outcome was detected.

To assess the efficacy in hypertensive emergencies in pregnancies, Elatrous et al [64] compared the effect of a 1 h infusion of labetalol with nicardipine in 60 patients with severe hypertension. Both drugs achieved target BP (20% lowering of BP) in the same proportion and at the same length of time.

Aya et al [65], using a loading dose of nicardipine of 1 mcg/kg/min in 20 pre-eclamptic patients (GA 29 - 36 weeks) obtained target BP (15% reduction in MAP) in all patients within 15-20 min. Treatment was followed by continuous infusion of a reduced dosage during one day to maintain MAP at 20-30% below the initial value. Foetal tolerance, assessed by foetal heart rate, was good, but severe tachycardia was noted in two patients.

Long-term treatment (31 - 250 days) with intravenous nicardipine (20 - 80 mg/day) in ten pregnant women with severe hypertension (both pre-eclampsia as pre-existing chronic hypertension) was shown to be efficacious and without maternal or foetal/neonatal adverse effects [66]. In conclusion, nicardipine seems to be a promising drug in the treatment of pre-eclampsia, but more data are needed on efficacy and safety aspects.

Other antihypertensive drugs

For treatment of an acute hypertensive emergency, hydralazin is most widely used. If hydralazin is, in rare cases, not effective, or, if the delayed onset of action of 10-20 min poses a risk to the mother, sodium nitroprusside can be used in acute situations in a dosage of 0.2-0.8 mg/min intravenously. Sodium nitroprusside is very potent and acts almost immediately by

direct dilatation of arterioles and veins. Its short duration of action (3-5 min) allows accurate titration of the blood pressure [29]. Due to the possible risk of accumulation of cyanide in foetus and mother, prolonged use is not recommended [26].

Several other classes of drugs are used to treat hypertension in non-pregnant patients, but are (relatively) contra-indicated during pregnancy. The use of angiotensin-converting enzyme inhibitors during pregnancy has been associated with foetal and neonatal renal failure, oligohydramnios, intrauterine growth retardation and increased foetal mortality [26]. The use of diuretics is contra-indicated in pre-eclampsia because plasma volume is already decreased and further volume depletion could affect the foetus adversely [29,67]. The beta-blocking agents metoprolol and atenolol are used orally as add-on treatment in severe pre-eclampsia. However, long-term use of beta-blocking agents has been associated with an increase in small-for-gestational-age infants, especially with atenolol [68]. Neonatal bradycardia, hypoglycemia and respiratory depression have also been reported as side effects, probably due to the beta-blocking effects.

Foetal and neonatal effects of maternal antihypertensive drug treatment

Maternal drug use may exert unwanted effects on the foetus by a direct pharmacological action of the drug after passing the placenta or by an indirect action, caused by compromising the uteroplacental perfusion. The latter can occur with all antihypertensive drugs through an overshoot of lowering of maternal blood pressure, resulting in foetal distress due to marginal placental function. Especially, the use of drugs which cause vasodilatation but which do not affect placental vascular resistance, such as (di)hydralazin, can cause a reduction in placental flow, leading to foetal distress. Indeed, a recent review [33] showed that hydralazin was associated with more adverse effects on foetal heart rate than other antihypertensive drugs. Drugs, which are effective at the level of the placental vasculature, such as calcium-channel blocking agents, should, theoretically, be able to maintain adequate placental perfusion.

Direct pharmacological effects are related to the amount of drug passing the placenta and the period of foetal exposure. Many drugs cross the placenta by simple diffusion, depending on the physicochemical characteristics of the drug and placental factors such as surface area and thickness of the membrane. An active transport mechanism across the placenta is also known to occur, mainly for endogenous substances such as amino acids and methyldopa. Recently, P-glycoprotein has been found to form a functional barrier between maternal and foetal blood circulation in the placenta, and use of P-glycoprotein inhibiting compounds may increase exposure of the foetus to P-glycoprotein substrates, such as digoxin [69].

As the use of antihypertensive drugs for management of pre-eclampsia is limited to the late second and third trimester, teratogenic effects are not relevant. However, in expectant management of pre-eclampsia, drugs are used in high dosages, often in combination treatment, for a prolonged period of time and information regarding placental transfer and

possible adverse effects on foetus and neonate are often unknown. This is even more relevant as many neonates born from mothers with severe early-onset pre-eclampsia are premature and are probably more susceptible to adverse drugs effects.

For most drugs mentioned in this review, information is available either from animal studies or from human data regarding placental transfer (Table 1.2). Almost all antihypertensive drugs used in pre-eclampsia, pass the placenta freely, but information regarding ketanserin and nicardipine are lacking.

Neonatal morbidity or mortality is usually reported in studies describing the use of antihypertensive drugs in severe pre-eclampsia but are hard to interpret due to the fact that inherent to the severity of the maternal illness, most children are born prematurely and growth-retarded, with a corresponding high morbidity and mortality in the first postnatal months. To date, only high maternal doses of labetalol have been associated in case reports with direct neonatal side effects (bradycardia, hypoglycaemia) [36,37].

Long-term follow up of neonates to assess possible effect of maternal antihypertensive drug use on growth and development is scarce. A six-year follow-up after maternal prophylactic oral use of ketanserin did no show any negative effect on mental development [70]. A follow-up study after use of dihydralazin for temporising management did not reveal any negative effect on morbidity and development of the infants in childhood [71]. For the other currently used antihypertensive drugs, no follow up studies are available.

Table 1.2 Transplacental transmission and foetal and neonatal effects of antihypertensive drugs used in pre-eclampsia.

Drug	Transplacental transmission	Foetal and neonatal effects
(Di)Hydralazin	High (equal to mother) [72]	Foetal distress due to acute drop maternal blood pressure[33]. No known adverse neonatal effects[71].
Methyldopa	High (equal to mother) [73]	No foetal and pediatric adverse effects known[26-28]
Labetalol	Intermediate (40-80% of maternal levels) [26]	Possible foetal intrauterine growth retardation. Beta blockade effects at birth (hypoglycemia, hypotension, bradycardia)[36,37]
Ketanserin	Unknown	No short or long term adverse effects in neonates reported[70]
Nifedipine	High (equal to mother) [74, 75]	In animal foetal acidemic responses, not confirmed in humans[48,49]
Nicardipine	Unknown	In animal foetal acidemic responses, not confirmed in humans[65,66]

AIMS AND SCOPE OF THE THESIS

Antihypertensive treatment is still the mainstay in the pharmacological approach of pre-eclampsia, to prevent maternal complications like cerebrovascular haemorrhage and organ damage. Well-known drugs such as oral methyldopa for mild pre-eclampsia and parenteral (di)hydralazin for severe pre-eclampsia are commonly used, but because of ambiguous reports on the safety of (di)hydralazin and its decreasing commercial availability, other potent, well studied, antihypertensive drugs are warranted.

This thesis focuses on efficacy and safety aspects of two newer antihypertensive drugs in pre-eclampsia: the serotonin antagonist ***ketanserin*** and the calcium-channel blocking agent ***nicardipine***.

To determine the efficacy of ketanserin in the population of severe early-onset pre-eclamptic patients, admitted to the Obstetric Intensive Care ward at the Erasmus MC, we performed a retrospective analysis of patients treated with ketanserin (**Chapter 2)**. Following our observation that a substantial proportion of our patients did not show an adequate antihypertensive response to ketanserin, we aimed to study pharmacological aspects in those patients.

To enable us to determine plasma levels of ketanserin, we developed a bioanalytical method (**Chapter 3**). We subsequently considered both pharmacokinetic aspects (**Chapter 4)**, as well as pharmacodynamic aspects (**Chapter 5)** of ketanserin in our effort to explain for the lack of antihypertensive efficacy of ketanserin.

To obtain more information regarding the safety of maternal use of ketanserin, we studied placental transfer, transfer into breast milk and disposition in the neonate after maternal treatment with ketanserin (**Chapter 6**). Knowing that 5-HT is one of the earliest neurotransmitters produced during foetal brain development, the possible influence on foetal 5-HT-receptor functionality after exposure to the 5-HT-receptor antagonist ketanserin was analysed in **Chapter 7**.

We studied the calcium-channel blocking agent nicardipine, as an innovative alternative treatment modality in early-onset pre-eclamptic patients, after treatment failure of either intravenous ketanserin or dihydralazin (**Chapter 8**)**.** We made an estimate of possible risks for the foetus by determining placental transfer and disposition of nicardipine into human milk **(Chapter 9).**

The interpretations of our results with recommendations for the respective roles of ketanserin and nicardipine in the treatment of pre-clampsia are discussed in the final chapter (**Chapter 10**).

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Chapter 2

Insufficient efficacy of intravenous ketanserin in severe early-onset pre-eclampsia

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ABSTRACT

The objective of the study was to analyze the efficacy of intravenous ketanserin in controlling blood pressure of severe early-onset pre-eclamptic patients. Pre-eclamptic patients (n = 47) with a gestational age (GA) between 21 and 32 weeks were treated with intravenous ketanserin in a maximum dosage of 14 mg/h, to obtain an intra-arterial diastolic blood pressure of 90 mmHg or below. The number of patients reaching and maintaining target blood pressure was retrospectively assessed. Patient characteristics associated with an adequate or inadequate response to ketanserin treatment were identified.

With a maximum intravenous dosage of ketanserin, target blood pressure was not achieved in 15 (32%) patients. A high systolic blood pressure at the start of treatment was significantly ($p = 0.02$) associated with failure of ketanserin treatment. The median period of ketanserin treatment in the responding group was 3 days (range 1-10 days). In 26 (55%) of initially successfully treated patients, additional antihypertensive drugs had to be added to maintain adequate blood pressure control.

In conclusion, intravenous ketanserin lacks antihypertensive efficacy in a substantial proportion of severe pre-eclamptic patients, despite high dosages. In patients who initially respond well to ketanserin treatment, additional antihypertensive treatment is often necessary to maintain adequate blood pressure control.

INTRODUCTION

The mainstay of treatment of pre-eclampsia is to lower the elevated blood pressure in order to prevent maternal complications like cerebrovascular hemorrhage and organ damage. In early-onset pre-eclampsia, adequate antihypertensive treatment can also yield sufficient time to administer antenatal steroids in order to improve neonatal outcome. In recent years, temporizing management with antihypertensive drugs is increasingly applied in patients with early-onset pre-eclampsia as long as both maternal and foetal condition permits [1-3].

The use of the serotonin (5-HT)- antagonist ketanserin for antihypertensive treatment of pre-eclampsia has increased in the past years. The drug acts by blocking the vasoconstrictive response upon binding of 5-HT to 5-HT_{2A} receptors in vascular tissue [4]. Because of its inhibitory effect on platelet aggregation, ketanserin is thought to have an additional beneficial effect in patients with HELLP-syndrome (haemolysis, elevated liver enzymes, low platelet-count) [5].

There are conflicting data on the efficacy of ketanserin in pre-eclampsia. Bolte et al [6] showed that ketanserin resulted in a better blood pressure control and less maternal side effects than dihydralazin. However, the dosage of dihydralazin was low and the patients treated with ketanserin needed antihypertensive co-medication to maintain adequate blood pressure control. Rossouw et al [7] compared short-term antihypertensive treatment with ketanserin and dihydralazin in a group of 80 patients with severe hypertension in the third trimester of pregnancy and found a better antihypertensive response with dihydralazin. However, ketanserin has the advantage of causing a more gradual decline in blood pressure than dihydralazin [6], which may reduce the risk of foetal distress. Ketanserin is one of the few drugs in the Netherlands licensed for pre-eclampsia in patients with a diastolic blood pressure of 110 mmHg or higher and has become the drug of choice to stabilize pre-eclamptic patients.

In 1999 we started using ketanserin as a first line treatment in our hospital for severe early-onset pre-eclampsia with the aim to control blood pressure and prolong pregnancy, as long as maternal and foetal condition allow. Because of the conflicting data on the efficacy of ketanserin and the need for clinical evaluation of the use of ketanserin in a population of severe early-onset pre-eclamptic patients, we performed a retrospectively analysis of the use of ketanserin, in terms of its efficacy in controlling blood pressure.

MATERIAL AND METHODS

Patients who were admitted to the antenatal High Care ward in the period 1999-2002 with severe early-onset pre-eclampsia (GA between 20 and 32 weeks on admission) were included in this evaluation. Severe pre-eclampsia was defined as the occurrence after 20 weeks of gestation of a diastolic blood pressure ≥ 110 mm Hg (Korotkoff V) and proteinuria ≥ 0.3 g/24h or the occurrence of a repetitive diastolic blood pressure > 90 mmHg in combination with

the HELLP-syndrome. HELLP-syndrome was defined as the simultaneous occurrence of ALAT and/or ASAT > 31 U/l (2 SD above the mean in our hospital), platelet count below 100 x 10^9 platelets/L and haptoglobin below 0.28 g/l (normal value 0.28-2.01 g/l).

Each woman received a radial arterial line for intra-arterial blood pressure measurement and a central venous line for central venous pressure (CVP) measurement.

Antihypertensive treatment was continued as long as foetal and/or maternal condition did not warrant delivery, as judged by the attending obstetrician. Foetal condition was assessed using cardiotocography (CTG) after a gestational age of 26 weeks or more.

Drug Treatment

Drug treatment was targeted at achieving an intra-arterial diastolic blood pressure of 90 mmHg or below (with a lowest limit of acceptance of 75 mmHg).

After a bolus injection of 5 mg, the infusion rate of ketanserin (Ketensin®, Pharmacia, Woerden, the Netherlands) was initiated at 4 mg/h and increased, according to the blood pressure, with 2 mg/h every 20 minutes to a maximum of 14 mg/h. Each increment was preceded by an intravenous loading bolus injection of 5 mg ketanserin. Patients, who were already taking oral antihypertensive drugs on admission (methyldopa and/or nifedipine) continued these medications at their established dosage regime.

The goal was to reach the desired intra-arterial diastolic blood pressure using the titration schedule with ketanserin to a maximum of 14 mg/h. If the desired blood pressure could not be obtained using the maximum dosage of ketanserin, oral antihypertensive drugs (methyldopa tot a maximum of 4 g daily or nifedipine to a maximum of 90 mg daily) and subsequently parenteral dihydralazin (starting with 1 mg/h to a maximum of 12 mg/h) or nicardipine (starting with 1 mg/h to a maximum of 10 mg/h) were started.

CVP was maintained at 5-6 mmHg, using pasteurized plasma-solution. All patients received antenatal steroids after 26 weeks of gestation.

Data Analysis

The efficacy of ketanserin treatment was assessed by analyzing the proportion of patients that reached the target blood pressure with ketanserin (responding group) and the group of patients that did not reach target blood pressure (non-responding group), despite the maximum dosage of ketanserin. The responding and non-responding groups were compared with respect to differences in initial patient characteristics (diastolic and systolic blood pressure and gestational age at the start of treatment), using the Mann-Whitney-U test. Additionally, both groups were compared with respect to the use of oral antihypertensive drugs at start of treatment and diagnosis of HELLP-syndrome at start of treatment, using the Chi-square test. Statistical analysis was performed using SPSS (version 10.1, SPSS Inc, Chicago, USA).

Safety of ketanserin treatment was determined as the absence of hypotensive periods during treatment (defined as an intra-arterial diastolic BP < 70 mmHg) as well as the assessment of

maternal adverse effects, as reported in the patient charts. Foetal and neonatal outcome was assessed in terms of intra-uterine foetal death (IUFD), neonatal death, number of severely growth restricted neonates at birth, pH-value umbilical artery, number of neonates with an APGAR score <7 at 5' and number of neonates admitted to ICU.

RESULTS

Forty-seven patients between 1999-2002 were admitted with severe early-onset pre-eclampsia to the high care obstetric ward and treated with intravenous ketanserin. Clinical characteristics of these 47 patients before start of treatment are presented in Table 2.1.

In 32 patients (68%) the target blood pressure was initially achieved, using ketanserin (responding group). In 15 patients (32%) the target blood pressure could not be achieved with ketanserin at the maximum dosage (non-responding group). In this latter group, three patients were subsequently delivered because of foetal distress, whereas in the other 12 patients adequate blood pressure control was achieved by adding (n=6) or increasing (n=6) the dosage of oral antihypertensive drugs (methyldopa, nifedipine) and subsequently adding intravenous dihydralazin (n=9, maximum dosage needed 12 mg/h) or nicardipine (n=2, maximum dosage needed 7 mg/h).

Table 2.1 Clinical characteristics of pre-eclamptic patients at the start of ketanserin treatment (n=47)

	Median (range) or number (%)
Maternal age (years)	31 (20-41)
Systolic blood pressure (mm Hg)	172 (140-240)
Diastolic blood pressure (mm Hg)	105 (91-125)
Proteinuria (g/24h)	2,62 (0,31-17,5)
HELLP syndrome (n)	23 (49%)
Gestational age (weeks)	28 (21-32)
Nulliparous (n)	37 (78%)
Twin pregnancy (n)	2 (4%)
Pre-existent hypertension (n)	7 (15%)
Oral co-medication before start of ketanserin:	
- Methyldopa	22 (47%)
- Methyldopa and nifedipine	3 (6%)
- Methyldopa and labetalol	1 (2%)
- Nifedipine	2 (4%)
- None	19 (41%)

[1] cause of death: respiratory distress associated with very preterm birth (n=2), cerebral bleeding (n=1) and cerebral lesions (n=1).

[2] cause of death: severe bronchopulmonary dysplasia

No relationship between failure of ketanserin therapy and median diastolic blood pressure at start of treatment [non-responding group 108 mmHg versus responding group 105 mmHg (p=0.35)] or gestational age at start of treatment [non-responding group 27 5/7 weeks versus responding group 28 6/7 weeks (p=0.6)] was demonstrated. A significantly (p=0.02) higher initial systolic blood pressure was found in the non-responding group (median 185 mmHg, range 145-240), as compared to that in the responding group (median 170 mmHg, range 140-190). In the group of non-responders seven (47%) patients did not use oral antihypertensive drugs before start of treatment as compared to 12 patients (37.5%) in the responding group (not significantly, p>0.1).

In 26 (55%) of the initially responding patients, additional antihypertensive drugs had to be started (n=9) or increased (n=17) during admission to maintain adequate blood pressure control. In seven patients, after encountering no safety problems with ketanserin, we decided to allow a maximum intravenous dosage of 0.2 mg/kg/h ketanserin (with an absolute maximum of 20 mg/h) and use bolus injections of 10 mg before each increment. In three of these patients, the higher dosage temporarily resulted in an adequate diastolic blood pressure, but in all patients additional antihypertensive treatment was necessary. Intravenous magnesium sulphate was administered to ten pre-eclamptic patients, to prevent eclampsia.

Overall, pregnancy was prolonged for a median of 6.5 days (range 0-40 days) after admission, using antihypertensive medication. The overall median period of ketanserin treatment in the responding group was 3 days (range 1-10 days). Foetal distress was most frequently (66%) the reason to deliver the patient. About 88% of the patients were delivered by caesarean section.

Twenty-three (49%) women were diagnosed with the HELLP-syndrome on admission. This high percentage can be attributed to the tertiary referral function of our Obstetric ward. Overall, in 11 (48%) of these women, the HELLP-syndrome resolved during treatment. In three women HELLP-syndrome developed during treatment. No significant differences were found in number of responders to ketanserin treatment between patients with HELLP-syndrome (18 of 23 patients, 78%) or without HELLP-syndrome (14 of 24 patients, 58%) at admission.

Maternal safety

Ketanserin was generally well tolerated. Some patients complained of fatigue, sedation and nasal stuffiness. Hypotensive periods were not observed during ketanserin treatment. Ketanserin has been associated with a risk of QT- prolongation and cardiac arrest [8]. In none of our patients, cardiac problems were apparent. However, obtaining an ECG was at that time not part of our standard procedures, so no reliable conclusions regarding QT-prolongation can be drawn.

Foetal and neonatal outcome are shown in Table 2.2. Although this study was primarily focused on maternal efficacy and most neonates were born from mothers who had been

using combinations of antihypertensive treatment before delivery, our data on perinatal mortality (24%) were comparable with historical data on perinatal mortality (20.5%, n=254) from our hospital of pre-eclamptic patients treated with dihydralazin [1].

Table 2.2 Foetal and neonatal outcome

	Median (range) or number (%)
Gestational age at delivery (weeks)	29 6/7 (24 5/7-34 4/7)
Number of fetuses (n)	49
Perinatal mortality (n)	12 (24%)
- Fetal death (n)	8 (16%)
- Neonatal death (n) (< 6 weeks)	41
- Infant death (n) (6 weeks- 1 year)	12
Birth weight (gram)	1030 (520-2340)
Growth percentile	
- Below 10% percentile (n)	18 (43%)
- Below 2.3% percentile (n)	3 (7%)
pH-value umbilical artery	7, 24 (6,96-7,38)
Apgar <7 at 5 min. (n)	9 (22%)
Admission to neonatal intensive care (n)	37 (90%)

DISCUSSION

Pre-eclampsia is treated with antihypertensive drugs to prevent maternal complications of high blood pressure and to yield sufficient time before delivery to administer steroids to the mother to improve the condition of the newborn. However, the intricate balance between lowering maternal blood pressure while not compromising foetal circulation, complicates administration of antihypertensive drugs to pre-eclamptic patients.

The current choice of antihypertensive drugs is limited. Ketanserin is one of the few drugs, licensed for treatment of pre-eclampsia in the Netherlands. This study describes our experiences with intravenous ketanserin in stabilizing blood pressure and maintaining adequate blood pressure control in patients with severe early-onset pre-eclampsia.

Our data show that one third of our patients did not respond to treatment with high doses of ketanserin with an adequate reduction of the blood pressure. This supports the suggestion in the literature that pre-eclamptic women treated with ketanserin are more likely to have persistent hypertension than those treated with other intravenous antihypertensive drugs, such as hydralazin [9].

The non-responding group warrants further analysis. An extended period of inadequate blood pressure control, caused by using a drug with no or insufficient effect may become critical for the patient with severe pre-eclampsia. We found that the diastolic blood pressure at the start of treatment did not show a significant relationship with failure of treatment, whereas the height of systolic blood pressure was a prognostic factor in determining the

efficacy of ketanserin treatment. The fact that in the responding group a (non-significantly) higher number of patients used oral antihypertensive drugs before start of treatment, might also have contributed.

Pharmacokinetic problems such as a subtherapeutic dosage or an ineffective concentration at the receptor site [10], may also cause an insufficient response. However, the fact that higher dosages of ketanserin did not elicit better responses, makes a dosage problem less likely. Another possibility is that the disease itself may account for different reactions of pre-eclamptic patients to treatment. The origin of pre-eclampsia is still largely unknown and the assumption that all pre-eclamptic patients can be considered as one group with a uniform disease may not be correct [11].

Over 50% percent of the patients in the responding group needed co-medication besides ketanserin during the treatment period to maintain adequate blood pressure control, which emphasizes the potency problem with ketanserin. However, since pre-eclampsia is a progressive disease, increasing antihypertensive treatment is usually warranted during admission. According to our protocol, second-line therapy after insufficient blood pressure control with ketanserin consisted of addition of oral antihypertensive medication and subsequently addition of parenteral dihydralazin or nicardipine. This protocol led to adequate blood pressure control in all patients and delivery in the majority of patients could be postponed.

Ketanserin is known to pass the placenta extensively [12] and pharmacological effects on foetus and neonate can therefore not be excluded. Analysis of CTG in our hospital of 23 pre-eclamptic patients during ketanserin treatment, compared to 40 dihydrazin treated patients (Lagro M et al, unpublished data) showed indeed that maternal ketanserin treatment decreases foetal heart rate, as compared to dihydralazin treated patients. The foetal and neonatal mortality data in this evaluation were comparable with historical data from our hospital, but valid conclusions regarding safety of ketanserin for the neonate cannot been drawn from this study, since most mothers had used a combination of antihypertensive drugs before delivery. However, other studies [13,14] have confirmed the lack of adverse effects in the neonate after maternal ketanserin use.

A retrospective observational and non-comparative study on the efficacy of a drug, as reported in this paper, has its limitations. Some of the patients, included in the analysis, already used co-medication on admission. Treatment was not blinded and the decision for any intervention may therefore have been biased by former experiences of the obstetrician.

However, this analysis was intended to evaluate our current policy in clinical practice of using ketanserin as a first-line treatment for severe, early-onset, pre-eclampsia. Despite the limitations of its methodology, our findings of a substantial group of non-responders on ketanserin are important for daily clinical care. More research is needed into alternative antihypertensive treatment strategies.

CONCLUSION

Intravenous ketanserin lacks antihypertensive efficacy in a substantial part of severe, early-onset pre-eclamptic patients, despite high dosages. A high systolic blood pressure at start of treatment was associated with an inadequate antihypertensive response on ketanserin. In patients who initially respond well to ketanserin treatment, additional antihypertensive treatment is often needed to maintain adequate blood pressure control.

Acknowledgements

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Chapter 3

Simultaneous quantitative analysis of ketanserin and ketanserinol in plasma by RP-HPLC with fluorescence detection

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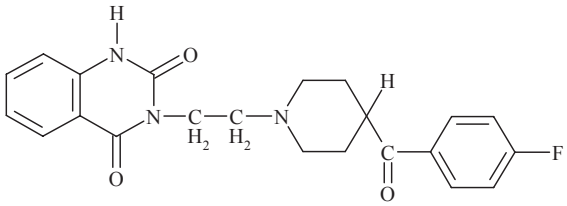
ABSTRACT

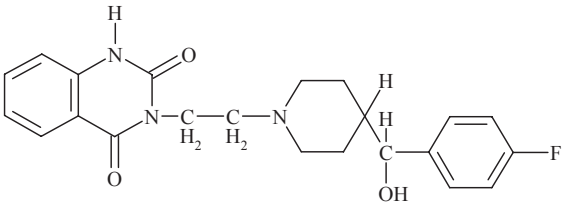
A sensitive and selective high-performance liquid chromatographic assay for the quantification of ketanserin and ketanserinol in human plasma was developed and validated. The procedure involves extraction of ketanserin and ketanserinol from plasma using an Extrelut NT-1 solid-phase extraction column. The chromatograph was equipped with a Hypersil BDS column (100 x 4.5 mm, 3 μm particle size). Separation was performed with a mixture of acetate buffer 0.01 M, pH 4.9 : methanol : acetonitrile (52 : 40 : 8, v/v/v). Detection was performed with fluorescence detection (λ_{ex} = 332 nm and λ_{em} = 410 nm). Calibration curves were linear (*r2 = 0.999*) in the range of 0 – 400 ng/ml for both ketanserin and ketanserinol. The repeatability coefficient for ketanserin and ketanserinol was 3.1 and 3.0%, respectively. The reproducibility coefficient for ketanserin and ketanserinol was 10.5 and 9.1%, respectively. The limit of quantification for both ketanserin and ketanserinol was 2.0 ng/ml. The mean recovery yield for both ketanserin and ketanserinol was 60%. In an 8 h work day approximately 60 samples, including calibration and reference standards could be processed.

INTRODUCTION

Ketanserin is a potent 5-HT_{2A} receptor antagonist with minor α_1-receptor blocking properties. The drug is metabolised in the liver by ketone reduction and oxidative N-dealkylation, and to a lesser extent in man by aromatic hydroxylation. The main metabolite is ketanserinol (R-46,742; reduced ketanserin; Figure 3.1) [1,2].



Ketanserin



Reduced ketanserin

Figure 3.1 Chemical structure of ketanserin and reduced ketanserin (ketanserinol).

Ketanserin has been used in the management of chronic hypertension. Recently, ketanserin has gained interest in the management of pre-eclampsia, a disease in which serotonin is thought to play a important part in the pathophysiology [3]. Several studies have shown that ketanserin is useful in the control of blood pressure in women with pre-eclampsia, but in severe pre-eclamptic patients, ketanserin shows variable efficacy [4]. This may be caused by insufficient dosage, resulting in subtherapeutic druglevels. Dosage regimens of ketanserin in the treatment of pre-eclampsia are not very well established [5]. Exploration of the pharmacokinetic properties of ketanserin in pregnant and pre-eclamptic patients, using plasma levels, is essential to design and implement rational dosage regimens. Furthermore, little information is available regarding the disposition of ketanserin in neonates after maternal treatment with ketanserin. Measurements of plasma levels in neonates are therefore needed to determine this disposition.

In the last two decades several methods have been published for the determination of ketanserin and/or its major metabolite ketanserinol in plasma. Some require UV detection and others fluorometric detection (Table 3.1). We experimented with several methods in our

laboratory, but none of the methods met our criteria on selectivity, specificity, sensitivity and throughput.

Also, measurement of plasma levels of ketanserin in neonates, who are usually premature or dysmature babies due to maternal pre-eclampsia, necessitated the search for a method that demanded only a small sample size. In order to overcome those limitations we developed an assay, quite different from those that already have been published. In this paper, a sensitive and selective method is described for the assessment of ketanserin and its reduced metabolite ketanserinol in small samples with a high throughput.

Table 3.1 Methods for the quantitative analysis of ketanserin and reduced ketanserin in biological matrices, applicable for clinical studies.

Method	Analyte	Matrix	Volume	Clean-up	LLQ	Reference
HPLC-UV	KT	P	1 ml	LLE	7.9 ng/ml	[6]
HPLC-FD	KT	P	1 ml	LLE	500 pg/ml	[7]
HPLC-UV	KT	P	1 ml	LLE	2 ng/ml	[8]
HPLC-UV	KT, KTOL	P	2 ml	LLE	10 ng/ml	[9]
HPLC-FD	KT, KTOL	P	1 ml	LLE	0.2 ng/ml	[10]
		U	0.2 ml			
HPLC-UV	KT	P	1 ml	LLE	-	[11]

HPLC, High-performance liquid chromatography; UV, ultraviolet detection; FD, fluoremetric detection; KT, ketanserin; KTOL, reduced ketanserin (ketanserinol); P, plasma; U, urine; LLE, liquid-liquid chromatography; LLQ, limit of quantification.

EXPERIMENTAL

Chemicals and reagents

Ketanserin(3-{2-[4-(4-fluorobenzyl)-1-piperidinyl]ethyl}-2,4-[1H,3H]-quinazolinedione, ketanserinol(3-{2-[4-(4-fluorobenzyl)hydroxymethyl-1-piperidinyl]ethyl}-2,4-[1H,3H]-quinazoline dione) and R-46,594 (used as internal standard, 3-{2-[4-(4-chlorobenzyl)-1-piperidinyl]ethyl}-2,4-[1H,3H]-quinazoline dione) were obtained from Janssen-Cilag (Beerse, Belgium). All organic solvents were of HPLC grade. Isoamyl alcohol, other reagents and chemicals were of analytical grade. Ammonia solution 25 % Suprapur, methanol, glacial acetic acid, sodium acetate and n-heptane were purchased from Merck (Amsterdam, The Netherlands). Acetonitrile was obtained from J.T. Baker (Deventer, The Netherlands).

Equipment

The chromatographic system consisted of a TSP P4000 HPLC pump, a TSP AS3000 automatic sampler (Thermo Separation Products Inc., San Jose, CA, USA), a Hypersil BDS C18 100 x 4.5 mm reversed-phase column packed with 3 μm particles (Chrompack, Middelburg, The Netherlands). Detection was performed with a Shimadzu RF-10A spectrofluorometric

detector (Shimadzu, Kyoto, Japan). The standard software for TSP equipment, TSP software PC1000 version 3.5.1, was used to record and integrate detector responses. The mobile phase consisted of 0.01 M acetate buffer, pH 4.9 : methanol : acetonitrile (42:40:8, v/v/v). The flow rate was set at 0.9 ml/min. Excitation and emission wavelengths were set at 332 nm and 410 nm, respectively.

Sample preparation and assay

A volume of 200 μL plasma was transferred to a 10-mL tube. Subsequently, 100 μL of methanol containing 53 ng of R46594 (internal standard) and 500 μl of 2.5 % ammonia solution was added. The aliquot was passed over a solid-phase extraction column (Extrelut NT-1, Merck KGaA, Darmstadt, Germany). The column was eluted with 7 mL n-heptane/isoamylalcohol 95/5. The eluent was evaporated to dryness under a gentle nitrogen stream at 60 °C. The residue was reconstituted in 100 μl of mobile phase. From this, 50 μl were injected into the HPLC system.

Plasma samples were stored at -20 °C before analysis. The influence of freezing and thawing on the levels of ketanserin in plasma was tested in triplicates. The levels of ketanserin were determined after one cycle of freezing at -20 °C and thawing, three cycles of freezing and thawing and six cycles of freezing and thawing. Results were compared with levels measured immediately after sampling in the patients.

Calibration graph

Standard solutions containing 0.06, 0.12, 0.3, 1.2, 3.0, 6.0 and 12.0 mg/L ketanserin and ketanserinol in methanol were prepared for the calibration graph and stored at 4 °C. Thereafter, 2.9 mL of blank plasma were spiked with 100 μL of standard solution.

Quantification was performed by calculating the peak-height ratios of each compound to the internal standard.

Recovery

The extraction yield of ketanserin and ketanserinol from plasma was calculated by comparing the detector response (peak height) of a spiked plasma sample with a detector response of a reference standard prepared in methanol, with the same concentration as the spiked plasma sample. The recovery was determined at seven different concentration levels and calculated as the ratio of peak height of plasma sample and peak height of reference solution, expressed as percentage. The spiked plasma samples and the reference standard solution were assayed in six fold.

Intra-day precision and accuracy

Inter- and intra-day precision and accuracy was calculated for the calibration curves. Spiked plasma samples with known concentrations of 2.0, 4.0, 10.0, 40.0, 100.0, 200.0 and 400.0

ng/mL ketanserin and ketanserinol were assayed. The measured concentrations were used to calculate the intra-day precision and accuracy. The standard deviation (SD) was used to calculate the intra-day precision, defined as:

$$\text{intra-day precision} = \frac{SD}{mean} * 100\ \% \quad (1)$$

The accuracy is defined as:

$$\text{accuracy} = \frac{\text{measured concentration} - \text{known concentration}}{\text{known concentration}} * 100\ \% \quad (2)$$

Inter-day precision

The spiked plasma samples that were used for the determination of the inter-day precision were assayed on four consecutive days. The measured concentrations were used to calculate the inter-day precision and accuracy.

Limit of quantification

The limit of quantification was determined by recording the noise of 30 injected blank plasma samples. The integrator calculated the noise. The limit of quantification was defined as 10 times noise level.

RESULTS

Chromatograms of a spiked plasma sample containing 100 ng/ml ketanserin and ketanserinol and 50 ng/ml internal standard [Figure 3.2 (A)], and a plasma sample after intravenous administration of ketanserin [10 mg/h; Figure 3.2 (B)] are presented. Evidently, no other plasma metabolites or endogenous compounds interfere with the measurement of ketanserin, ketanserinol and internal standard. The total run time was 10 min. The retention times of ketanserin, ketanserinol and internal standard are 3.0, 4.4 and 8 min, respectively. In a standard working day about 60 samples could be processed, including calibration and reference standards. Samples can be run overnight, using an automatic sampler device.

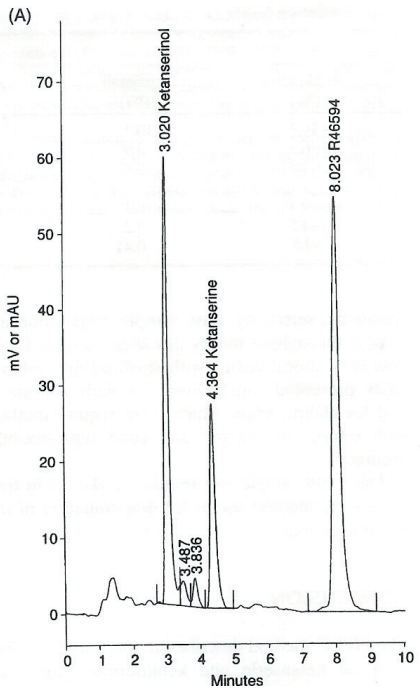


Figure 3.2 (A)
Chromatogram of plasma sample spiked with 100 ng/mL ketanserin and ketanserinol and 50 ng internal standard

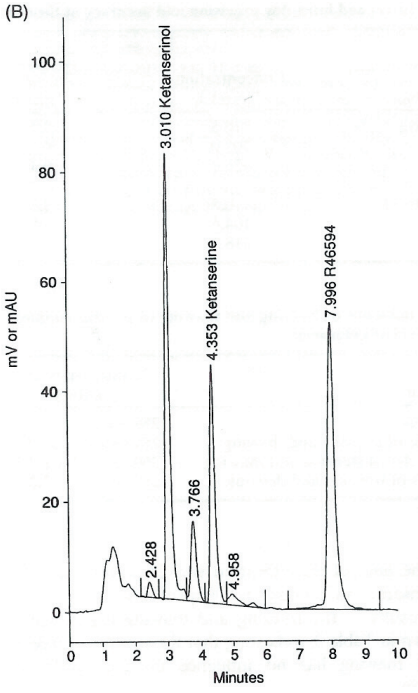


Figure 3.2 (B)
Chromatogram of patient plasma sample after continuously intravenous administration of ketanserin

The calibration curves of peak height for ketanserin and ketanserinol were linear in the, clinical relevant, concentration range of 10 - 400 ng/ml (r^2 = 0.999). The inter-day, intra-day precision and accuracy are listed in Table 3.2. The recovery yield of ketanserin and ketanserinol was 60%. The limit of quantification was 2.0 and 1.7 ng/ml for ketanserin and ketanserinol, respectively.

Table 3.2 Inter-day-, intra-day precision and accuracy at three different concentration levels

Compound	Concentration	Intra-day		Inter-day	
	[ng/ml]	Precision [%]	Accuracy [%]	Precision [%]	Accuracy [%]
Ketanserin	10.5	3.1	-14.1	10.5	-5.1
	105.0	0.5	-6.3	3.9	-5.8
	420.0	0.4	-1.8	0.4	-1.6
Ketanserinol	10.5	3.0	-9.1	9.1	-0.5
	104.6	1.0	-4.5	3.2	-2.2
	418.3	0.4	-1.8	0.41	-1.8

The results of the freezing and thawing experiments are shown in Table 3.3, indicating that the process of freezing and thawing has no influence on the levels of ketanserin.

Table 3.3 Influence of freezing and thawing of plasma samples on the level of ketanserin

Condition	Ketanserin level (ng/ml)
No storage	259 ± 21.4
One cycle of freezing and thawing	254 (98.1%) ± 3.5
Three cycles of freezing and thawing	260 (100.3%) ± 7.5
Six cycles of freezing and thawing	259 (100%) ± 3.2

DISCUSSION

In developing a sensitive and selective assay for ketanserin and ketanserinol, we have tried liquid-liquid extractions with several organic compounds, but this yielded very low recovery of ketanserin and ketanserinol from plasma. In addition, endogenous compounds were interfering in the assay.

The use of Extrelut NT-1 solid-phase extraction columns allows very simple and quick sample preparation without the need for further sample purification. Interfering peaks became negligible when these solid-phase extraction columns were used. Furthermore, relatively high and reproducible extraction yields of ketanserin and ketanserinol from plasma were obtained with solid-phase extraction.

Fluorimetric detection was chosen because of both its sensitivity and selectivity for the assessment of ketanserin and ketanserinol in plasma samples. We also conducted this assay with UV-detection. However, chromatograms obtained with UV detection gave poor peaks, even for the highest calibration concentrations.

The method described in this paper can be easily implemented in other laboratories. The combination of sensitivity, selectivity, easy sample preparation and high sample throughput makes this assay suitable for application in a clinical setting with small sample volumes. The assay presented can be used for daily routine analysis and for clinical trials, which often require methods with high sample throughput and good reproducibility and accuracy.

The small sample size required (200 μl) in this assay, makes the method useful for determination of drug levels in neonates.

CONCLUSION

The HPLC method described in this paper for the assessment of ketanserin and ketanserinol with solid-phase extraction and fluorimetric detection in plasma allows a selective and sensitive analysis of these compounds without interference from endogenous compounds. The entire procedure including sample preparation, extraction and HPLC determination can be easily performed in a standard routine laboratory.

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Chapter 4

Population pharmacokinetics of ketanserin in pre-eclamptic patients and its association with antihypertensive response

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ABSTRACT

Ketanserin is an antihypertensive drug that is increasingly being used parenterally in the treatment of pre-eclampsia. Because of lack of efficacy in a substantial part of our pre-eclamptic patients, we determined plasma concentrations of ketanserin in 51 pre-eclamptic patients. Population pharmacokinetic parameters were assessed using the iterative two-stage Bayesian population procedure. The influence of individual pharmacokinetic parameters on antihypertensive response, expressed as the attainment of a diastolic blood pressure ≤ 90 mmHg using ketanserin treatment, was analysed.

Almost all plasma concentrations of ketanserin were in or above the therapeutic range. The individual pharmacokinetics of ketanserin in pre-eclamptic patients showed an accurate fit using a three-compartment model.

The pharmacokinetic parameters in our pre-eclamptic population were a metabolic clearance (Cl_m) of 37.9 ± 10.86 L/h and a volume of distribution (V1) of 0.544 ± 0.188 L/kg, which is comparable with data from healthy volunteers.

Despite a considerable interindividual variation, no correlation was found between differences in pharmacokinetic parameters and antihypertensive response.

We conclude that therapeutic plasma levels can be obtained in pre-eclamptic patients with a fixed dosage schedule of ketanserin. Differences in antihypertensive responses within a pre-eclamptic population cannot be attributed to pharmacokinetic differences.

INTRODUCTION

Pre-eclampsia is the major cause of morbidity and mortality in pregnant women worldwide. The disease is characterised by a rise in blood pressure and proteinuria after the 20th week of gestation. Untreated, pre-eclampsia can lead to severe organ damage, including liver, kidneys and/or brain. Endothelial dysfunction caused by unknown factors produced by the placenta is thought to be the main pathophysiological factor causing the disease [1] and the only cure is delivery. However, in early-onset pre-eclampsia, the resulting morbidity and mortality for the premature neonate is high.

In recent years, intensive management of the early-onset pre-eclamptic patient, using parenteral antihypertensive drugs, has shown an improved outcome for the neonate [2-5].

To obtain adequate blood-pressure control in pre-eclamptic patients, the ideal antihypertensive drug should be potent, safe for mother and foetus and easily titrated, according to the blood pressure. The drug used most often is hydralazine (or its analogue dihydralazin), an antihypertensive drug acting directly on the vessel-wall. (Di)hydralazine has the advantage of an extensive experience and a good efficacy. However, administration of the drug needs to be accompanied with adequate volume expansion and monitoring and the drug causes frequently maternal adverse effects like reflex tachycardia, headache and nausea. In several European countries, the drug is not commercially available anymore.

The antihypertensive drug ketanserin has been recently licensed in the Netherlands for treatment of pre-eclampsia. Pharmacologically, the drug acts as an antagonist of the 5-HT_{2A} receptor in the blood vessel, counteracting the vasoconstrictive response on serotonin [6]. The drug is orally and parenterally available and causes few side-effects. Its positive effect on platelet aggregation and thrombus formation is considered an advantage in patients with the HELLP-syndrome (haemolysis, elevated liver enzymes, low platelets) [7].

However, the drug appears to lack efficacy in severe early-onset pre-eclamptic patients. In a subgroup of patients, the drug shows minor antihypertensive effects and in a substantial part of the patients additional oral antihypertensive drugs or parenteral dihydralazine is needed to obtain adequate blood pressure control [8,9].

Van Schie [10] suggested that an individual dosing problem might cause the lack of efficacy. Currently, a standard dosage schedule is applied, independent of patient characteristics, such as body weight, amount of oedema and liver function. However, a population of pre-eclamptic patients can differ greatly with respect to these characteristics. Differences in these patient characteristics may result in differences in drug levels at the receptor site and influence efficacy.

The aim of this study was to analyse the pharmacokinetic parameters of ketanserin within a pre-eclamptic population and to assess whether differences in pharmacokinetic parameters are related to variations in antihypertensive response to this drug.

MATERIALS AND METHODS

Study population

Pre-eclamptic patients, admitted to our antenatal ward and treated with ketanserin, were included in the study in the period between 1999-2001. Severe pre-eclampsia was defined as the occurrence, after 20 weeks of gestation, of a diastolic blood pressure ≥ 110mm Hg and proteinuria ≥ 0.3 g/l during a 24-h urine collection, or the occurrence of repetitive diastolic blood pressure > 90 mmHg in combination with the HELLP-syndrome.

The patients received ketanserin according to a standard dosing schedule. Drug treatment was targeted at an intra-arterial diastolic blood pressure of ≤ 90 mmHg. In case of an inadequate blood pressure control with ketanserin, oral antihypertensive drugs such as nifedipine of methyldopa or parental dihydralazin were added. Treatment was continued until foetal or maternal condition warranted delivery. The Medical Ethical Committee of our hospital approved the study and all patients gave written informed consent.

Treatment

The standard dosing schedule was as follows: all patients started treatment within an hour after admission, with a bolus injection of 5 mg ketanserin, followed by a continuous infusion of 4 mg/h. The dosage was titrated according to the blood pressure by increments of 2 mg/h to a maximum of 14 mg/h. Each increment was preceded by a bolus of 5 or 10 mg.

In 2001, after encountering no safety problems, the maximum dosage was increased to a maximum of 20 mg/h. A venous blood sample was drawn before each bolus injection. During treatment periods without dosage changes, a daily blood sample was drawn.

Analytical techniques

The plasma levels of ketanserin were assessed using a newly developed validated reversed-phase high performance liquid chromatographic assay with fluorescence detection [11]. The limit of quantification for ketanserin was 2.0 ng/ml. The repeatability coefficient was 3.1% and the reproducibility coefficient was 10.5 % for the concentration range 0-400 ng/ml.

Data analysis

Population pharmacokinetic parameters were calculated using the iterative two-stage Bayesian population procedure of the pharmacokinetic program MwPharm (MW Pharm version 3.5 0, Mediware, Groningen, the Netherlands) [12].

The model used to describe ketanserin pharmacokinetics was based on a three compartment pharmacokinetic model, as described by Heykants [13] for adult male subjects, with elimination from the central compartment. The values of this model (as shown in Table 4.1) were used as initial values in our study.

For each patient, characteristics (age, body weight, serum creatinine, dosage regime, plasma ketanserin levels) were entered into MwPharm. Subsequently, individual pharmacokinetic parameters were calculated by maximum a posteriori Bayesian fitting [14], applying a log-normal distribution parameter.

The response to ketanserin treatment was considered effective if a diastolic blood pressure of ≤ 90 mmHg was obtained using the standard ketanserin dosage schedule. If additional antihypertensive treatment had to be added at the maximum dosage level of ketanserin in order to obtain adequate blood pressure control, the treatment was considered a failure.

Statistical analysis

The pharmacokinetics parameters were compared between the pre-eclamptic population and data from literature regarding volunteers and other patient groups. Responders and non-responders within the pre-eclamptic population were analysed with respect to the pharmacokinetic parameters, using the Students t-test. A p-value < 0.05 was considered statistically significant.

RESULTS

Fifty-one patients (age ranging between 21 and 41 years) were included in our study, with a gestational age at admission of 28 weeks (range 21-39). The median duration of ketanserin treatment was 4.8 days (range 0.1-38). Diastolic blood pressure at admission ranged between

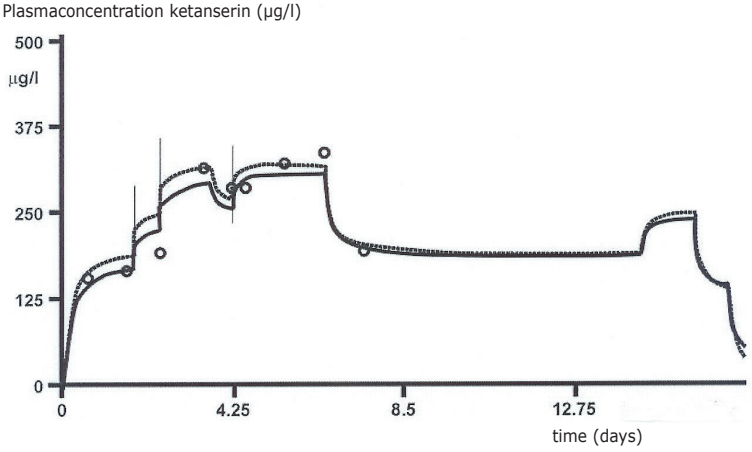

Figure 4.1 Example of plasma concentration - time data of a pre-eclamptic patient, treated with intravenous ketanserin. The circles represent actually measured plasma-concentrations, the straight line is the plasma-concentration curve based on initial population data and the dotted line is the fitted curve.

90-140 mmHg (median 100 mmHg), and corresponding systolic bloodpressure at admission ranged between 135-240 mmHg (median 172 mmHg).

A total of 345 blood samples were collected and a concentration range of ketanserin of 3 to 889 ng/ml was determined. For each patient a median of 6 (range 1-25) blood samples was obtained. Only five plasma concentrations (1%) were below the therapeutic range for ketanserin of 15-140 ng/ml as defined in literature [15]. Two of these samples were taken after infusion of ketanserin was stopped. In one patient, who received ketanserin in a low dosage of 2 mg/h due to border-line blood pressures, three samples were below the therapeutic range. A total of 134 samples (39%) fell within the therapeutic range and 206 samples (60%) were above the therapeutic range. Plasma drug concentration-time data showed satisfactory fits when the three-compartment model (Figure 4.1) and the Bayesian fitting procedure with an estimated log-likelihood value of 48.498 were applied.

The estimated population pharmacokinetic parameters of our pre-eclamptic patients are shown in Table 4.1. A wide variation, mainly in metabolic clearance, was found in our population of pre-eclamptic patients.

Table 4.1 Initial [13] and pre-eclamptic population pharmacokinetic parameters (n=51).

	Cl_m (l/h)	V1 (l/kg)	k12 (/h)	k21 (/h)	k13 (/h)	k31 (/h)
Initial	33 ± 3.3	0.719 ± 0.192	5.79 ± 6.97	3.43 ± 2.52	0.213 ± 0.121	0.087 ± 0.08
Pre-eclamptic	37.9 ± 10.86	0.544 ± 0.188	140.85 ± 54.27	47.12 ± 11.12	0.43 ± 0.313	0.052 ± 0.012

Results are expressed as mean (± standard deviation) Cl_m, metabolic clearance; V1, volume of distribution in central compartment, normalised to body weight; k12, rate constant from the central compartment to the peripheral compartment; k21, rate constant from the peripheral compartment to the central compartment; k13, rate constant from the central compartment to the deep tissue compartment.

In 16 of the 51 patients (31%), ketanserin treatment did not result in adequate blood pressure control and additional antihypertensive treatment was added *(non-responding group)*. The metabolic clearance and volume of distribution for each individual responding and non-responding patient is shown in Figure 4.2A and 4.2B. Mean pharmacokinetic parameters of the response group versus the non-responding group are summarized in Table 4.2. No significant relationship between treatment- response and clearance or volume of distribution could be established.

Table 4.2 Pharmacokinetic parameters of ketanserin in the non-responding pre-eclamptic patients (n=16) versus the responding pre-eclamptic patients (n=35). Results are expressed as mean (± standard deviation)

	Cl_m (l/h)	V1 (l/kg)	k12 (/h)	k21 (/h)	k13 (/h)	k31 (/h)
Non-responders	35 ± 5.8	0.58 ± 0.167	138 ± 17.5	47 ± 3.3	0.51 ± 0.439	0.052 ± 0.0024
Responders	40 ± 8.3	0.55 ± 0.097	146 ± 35	47 ± 3.6	0.50 ± 0.374	0.052 ± 0.0036

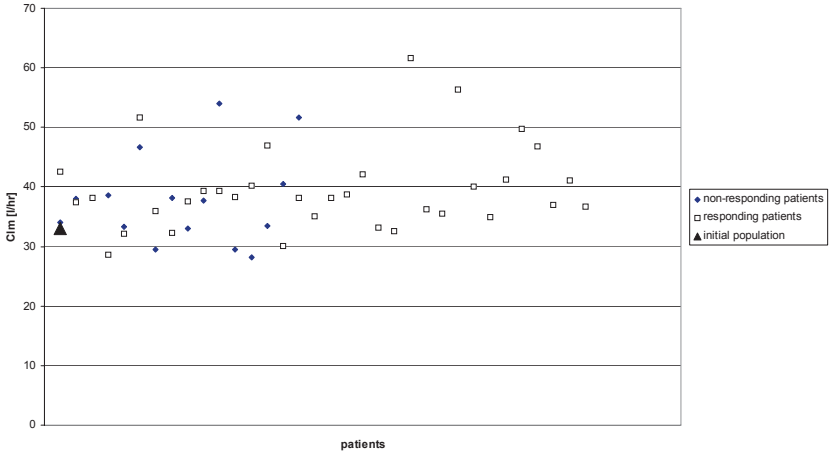

Figure 4.2A Metabolic clearance (Cl_m) of responding and non-responding patients

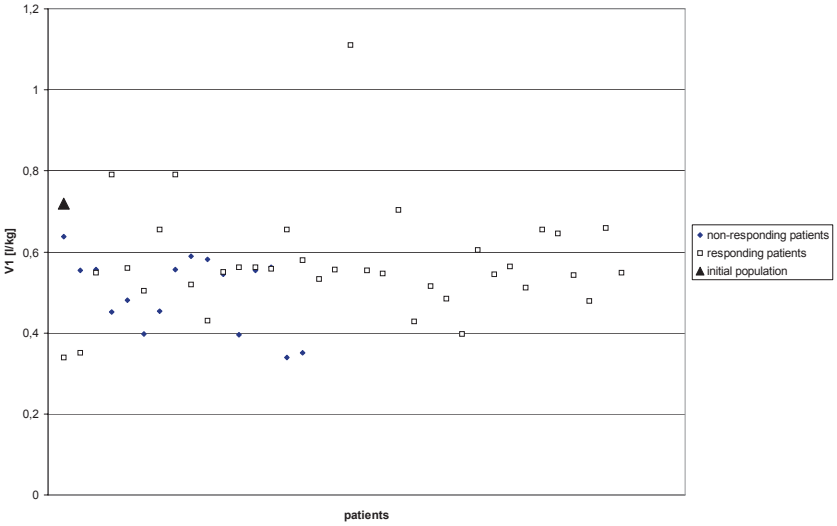

Figure 4.2B Volume of distribution (V1) of responding and non-responding patients

DISCUSSION

The serotonine antagonist ketanserin is an antihypertensive drug, which is increasingly being used in pre-eclamptic patients. Pharmacokinetic data of ketanserin have been studied well in other populations [15-17] but data in pregnant women as well as data in pregnant pre-eclamptic patients are lacking, while these populations are known to differ greatly in comparison to healthy volunteers with respect to parameters as presence of oedema, hepatic function, renal function and albumin level. Depending on the severity of the disease, individual differences within a pre-eclamptic population can be substantial [18]. The currently used fixed dosage schedule of ketanserin is based on limited studies and might not be appropriate for all pre-eclamptic patients. Indeed, data show that ketanserin appears to lack efficacy in a substantial part of pre-eclamptic patients [8,9]. We hypothesized that these differences in efficacy of ketanserin treatment were caused by pharmacokinetic differences.

We found therapeutic and supratherapeutic plasma levels of ketanserin in 99% of our samples, indicating that the current dosage-schedule results in pharmacological plasma concentrations in almost all of the patients.

To analyse the individual plasma concentrations versus administered dosages, we used a three compartment pharmacokinetic model. A pronounced distribution of ketanserin to tissues takes place after administration, even more extensive than binding to plasma proteins (extra cellular fraction bound = 94%, [19]). Ketanserin has been shown to metabolise by keton reduction and oxidative N-dealkylation to its main metabolite ketanserinol [20]. Mean sequential half-lives of ketanserin were described as 0.129, 1.98 and 14.3 h [13].

The metabolite ketanserinol is excreted mainly in urine. Its affinity for 5-HT_{2A} receptor is approximately 1000-fold lower than the parent drug, but a reduction-oxidation equilibrium seems to exist between ketanserin and ketanserinol [20]. The ketanserinol pool has been considered an adjacent metabolite compartment in the multicompartment character of disposition of ketanserin and it is thought to be reversibly connected with the central ketanserin compartment. Our data for ketanserin in individual pre-eclamptic patients showed a good fit with the three-compartment model, described in literature [13].

The pharmacokinetic parameters, found in the study, are comparable with the data found in literature in healthy non-pregnant volunteers [19,21]. The metabolic clearance of 37.9 L/h ± 10.86 is slightly higher than the clearance from the initial population model and data from other authors on healthy non-pregnant volunteers (Cl_m: 24.6 L/h ± 3.72 [19] and 26.7 L/h ± 4.8 [21]. Metabolic clearance of ketanserin is primarily dependent on hepatic flow, enzymatic activity and fraction of unbound drug [17]. A pre-eclamptic population is characterised by a usually low but very variable level of plasma-albumin. This might result in a substantial increase in the fraction of unbound drug, leading to a higher metabolic clearance, which is seen in our patients. This hypothesis needs to be confirmed by determination of unbound versus

protein-bound drug. Unfortunately, the detection limit of our current chromatographic assay did not allow for accurate measurements of the unbound drug-concentration.

The values for the intercompartmental rate constants k21 and k12 differed widely from the initial population pharmacokinetic values. Ideally, these intercompartmental rate constants are best fitted based on samples taken during the distribution period. For ketanserin, the distribution phase takes place within minutes, whereas most blood samples in our study were taken after 20 min of more after the dosage change. This will have influenced the reliability of assessment of k21 and k12 in our study.

We could not detect a relationship between antihypertensive response and individual variations in Cl_m or V1. The main part of the plasma concentrations measured was in or above the therapeutic range of 15-140 ng/ml. These findings suggest that the differences in antihypertensive response might not be attributed to pharmacokinetic differences within a population but might be more related to pharmacodynamic aspects, like 5-HT_{2A} receptor activity within a pre-eclamptic population or to differences in the severity of the disease pre-eclampsia itself.

Our own limited experience in increasing the dosage of ketanserin to a maximum of 20 mg/h did not elicit better responses in the main part of these patients. However, the very rare but potentially dangerous QT prolongation, associated with use of ketanserin, might be correlated with high dosages of ketanserin [22] and therefore increasing the dosage of ketanserin cannot be done indiscriminately. Furthermore, maternal ketanserin use has shown to result in a high transplacental transmission of ketanserin [23] and although no negative effects on the neonate has been observed so far, pharmacological effects in the neonate cannot be excluded.

CONCLUSION

Therapeutic concentrations of ketanserin were obtained in all patients after a fixed dosage schedule of ketanserin. Pharmacokinetic parameters of the pre-eclamptic population were comparable to healthy volunteers. Individual variations in Cl_m and V1 could not explain the lack of antihypertensive response in one-third of the patients.

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Chapter 5

Functional reactivity of 5-HT receptors in human umbilical cord and maternal subcutaneous fat arteries after normotensive or pre-eclamptic pregnancy

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ABSTRACT

The functional reactivity of 5-hydroxytryptamine (serotonin; 5-HT) receptors in foetal umbilical cord arteries (UCA) and maternal subcutaneous fat resistance arteries (SFA) was investigated in normotensive and pre-eclamptic pregnancy. Segments of UCA and SFA were mounted in tissue baths and concentration-response curves to 5-HT and sumatriptan (5-$HT_{1B/1D}$ receptor agonist) were constructed in the absence or presence of ketanserin (5-HT_{2A} receptor antagonist) or GR125743 (5-$HT_{1B/1D}$ receptor antagonist). Both 5-HT and sumatriptan contracted all UCA segments studied. The responses to 5-HT and the potency of ketanserin in UCA were not different between the study groups, indicating a similar profile of the 5-HT_{2A} receptor. In contrast, the potencies of sumatriptan and GR125743 were significantly higher in normotensive full-term pregnancies than in normotensive pre-term pregnancies in UCA. The response to sumatriptan in UCA arteries was not significantly different between pre-eclamptic and normotensive pregnancies. However, the potency of both sumatriptan and GR125743 were positively correlated to the gestational age in the normotensive group, while this relationship was absent in the pre-eclamptic group. In SFA, responses to 5-HT and sumatriptan were not different between the pre-eclamptics patients and normotensive controls.

In conclusion, in both UCA and SFA, 5-$HT_{1B/1D}$ and 5-HT_{2A} receptors mediate vasoconstriction. The sensitivity of 5-$HT_{1B/1D}$ receptors increases in the last trimester in the UCA in normal pregnancies, which seems to be expedited in pre-eclamptic patients. Hence, further studies on 5-$HT_{1B/ID}$ receptors may give new insights into the foetal development and pathophysiology of pre-eclampsia.

INTRODUCTION

Pre-eclampsia affects about 5% of all pregnancies and is a major cause of morbidity and mortality to both mother and foetus [1]. The etiology of pre-eclampsia is still largely unknown, although it has been linked to abnormalities in trophoblast invasion into the placental bed [2]. The syndrome is clinically characterized by maternal hypertension and proteinuria, and diagnosed after 20 weeks of gestation. The usual physiologic adaptations in response to increased fluid volume that are observed in normal pregnancies are attenuated in pre-eclampsia, resulting in increased vascular resistance [3]. This higher vascular resistance of the maternal vasculature in pre-eclampsia may be attributed to increased plasma concentrations of contractile agents [4-7], but could alternatively be attributed to an increased sensitivity of the arteries to vasoconstrictor agents like angiotensin II [8-9], 5-hydroxytryptamine (5-HT; serotonin)[7] and noradrenaline [10], combined with a decreased response to vasodilating peptides such as calcitonin gene-related peptide [11] and acetylcholine [12]. Vascular hyperreactivity in pre-eclamptic women has been demonstrated by, for example, an increased vasoconstrictive response to the cold pressor test, which is mediated by α-adrenergic receptors [13]. Obviously, increased plasma levels of a vasoconstrictor, in combination with an increased sensitivity of the respective receptors, may synergistically lead to increased vascular resistance and hence hypertension.

It is well established that in pregnancy the umbilicoplacental circulation lacks autonomic innervations [14] and thus, the regulation of vascular reactivity is mainly dependent on local autocrine and circulating vasoactive substances in the blood. 5-HT potently constricts human umbilical blood vessels [15] and causes platelet aggregation, which leads to further release of 5-HT; both these properties may synergistically impede normal placental blood flow in conditions like pre-eclampsia [7]. 5-HT receptors have been classified into 7 main groups (5-HT_1 - 5-HT_7 receptors) with several established subtypes in the first two groups [16]. Studies in umbilical cord reveal that the contractile response to 5-HT involves 5-HT_{1B} and 5-HT_2 receptors [17]. 5-HT_7 receptors are also believed to mediate relaxation in human arteries [18], but information regarding their role in the regulation of hemodynamics in umbilicoplacental vessels is lacking. Maternal circulatory concentrations of 5-HT are significantly increased in women with pre-eclampsia [5-7]. High levels of circulating factors like 5-HT in pre-eclampsia may induce endothelial dysfunction in the resistance arteries of the mother [19], where 5-HT acts as a potent vasoconstrictor [20], which may further contribute to the increased vascular resistance observed in pre-eclampsia. In addition, the increased plasma levels of 5-HT may also affect 5-HT receptors in umbilicoplacental and maternal resistance vessels. On this basis, the present study was undertaken to study the differences in the functional reactivity of 5-HT receptors between normotensive pregnant and pre-eclamptic women.

We studied umbilical cord arteries (UCA) as a representative of foetal blood vessels and subcutaneous fat arteries (SFA), representing maternal resistance arteries.

METHODS

Umbilical cord and subcutaneous abdominal fat (in case of caesarean section) were obtained after informed consent from pregnant women admitted to the Obstetrics Department of Erasmus MC, St. Franciscus Gasthuis or Ikazia Ziekenhuis (all Rotterdam, The Netherlands). Pre-eclamptic patients (diastolic blood pressure > 90 mmHg and protein/creatinine ratio (PCR) > 30 mg protein/mmol creatinine), as well as normotensive females of different gestational ages were included in the study. Patients were classified as pre-term at a gestational age < 37 weeks; all patients with higher gestational age were classified as full-term. The pre-eclamptic patients were treated with different antihypertensive drugs like alpha-methyldopa, dihydralazine, ketanserin, labetalol and nicardipine. Women suffering from diabetes and normotensive women with intra-uterine growth retardation (IUGR, birth weight is < 10^{th} percentile weight for his/her age in weeks corrected for gestational age, parity and foetal sex [21]) and in the pre-eclamptic group patients suffering from pre-existing hypertension were not included in the study. The Ethics Committee of the Erasmus MC approved this study.

Segments of umbilical cord and maternal subcutaneous fat tissues were collected in cold Krebs bicarbonate solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.1; pH 7.4), transported to laboratory and stored in carbogenated (95% O_2 and 5% CO_2) Krebs bicarbonate solution at 4˚C. UCA were isolated from the umbilical cord after removing the Wharton's jelly and subcutaneous arteries were isolated after removing adhering subcutaneous fat. Functional experiments were performed on the same or the subsequent day. In pilot experiments, we did not observe any differences in responses of UCA to KCl, 5-HT or sumatriptan between experiments that were performed on the same day or the next day.

UCA segments of 3-4 mm length (internal diameter: 1.5-2.0 mm) were suspended with the help of stainless-steel hooks in 15-ml organ baths. These segments were set at a pretension of 25 mN as determined to be the optimal tension in pilot experiments (data not shown). Segments showing bulging or macroscopic undulations were not used in experiments. SFA segments were cut into rings of 1-2 mm length with an internal diameter of 150-500 μm. Artery segments were suspended in Mulvany myographs on two parallel titanium wires. Subsequently, the distance between the wires was normalized to $0.9 \times I_{100}$ (I_{100} is the distance between the pins when the transmural pressure equalizes 100 mm Hg) to achieve optimal conditions for active force development. For both UCA and SFA, the vessel segments were continuously bubbled with 95% O_2 and 5% CO_2 and the temperature was maintained at 37˚C. After an initial equilibration period of 45 min, two successive challenges with KCl (30 mM) were performed to verify the reproducibility of the response. Subsequently, KCl (100 mM) was added to determine the reference contractile response of the artery segments to compensate for small differences in the muscle mass of the artery segments. In both UCA and SFA, endothelial function was evaluated by observing the relaxant response to substance P (100

nM) after precontraction with U46619 (9,11-dideoxy-11α, 9α-epoxy, methanoprostaglandin $F_2\alpha$ 10-300 nM). Cumulative concentration response curves to 5-HT were constructed in a parallel setup in the presence of vehicle (saline) or after 30-min incubation with the 5-HT_{2A} receptor antagonist, ketanserin (10 nM, 100 nM or 1 μM). Similarly, concentration response curves to sumatriptan were constructed in a parallel setup in the presence of vehicle or three increasing concentrations (10 nM, 100 nM or 1 μM) of the 5-$HT_{1B/1D}$ receptor antagonist, GR125743 (N-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl).[1,1-biphenyl]-4-carboxamide hydrochloride) [22].

In UCA, we also investigated the relaxant response to 5-HT mediated by putative 5-HT_7 receptors. For discerning 5-HT_7 responses in UCA, artery segments were incubated with both ketanserin and GR125743 (100 nM, each), the segment was precontracted with KCl (30 mM) and then 5-HT was added in a cumulative manner. Due to the limited number of vessel segments that could be isolated from the small samples of subcutaneous fat that were obtained during caesarean sections, experiments were performed using only one concentration (100 nM, each) of ketanserin and GR125743 and no experiments on the role of 5-HT_7 receptors were performed. In both UCA and SFA, only single concentration responses curve was constructed in each artery segment.

Chemicals

5-HT, U46619, substance P (Sigma Chemicals Co., Steinheim, Germany), KCl (Merck, Darmstad, Germany), ketanserin tartrate (Janssen, Beerse, Belgium), sumatriptan succinate (GlaxoSmithKline, Stevenage, U.K.) and (N-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamidehydrochloride) (GR125743) (Pfizer Limited, Sandwich, Kent, U.K.) were all dissolved in distilled water and stored in aliquots at –80 ˚C.

Statistical analysis

The contractile response to 100 mM KCl, expressed in milli-Newton (mN), was used to compare the contractile force developed by arteries from different experiment groups. All contractile responses to the agonists are expressed as percentage of the contraction induced by 100 mM KCl. All values are expressed as mean ± S.E.M. and n represents the number of segments, each segment obtained from a different patient. Since it was not always possible to study all compounds in segments obtained from one patient, the number of segments for each experimental condition may differ from the total number of patients in a group. Concentration response curves were analyzed using nonlinear regression analysis using GraphPad Prism 3.01 (GraphPad Software Inc., San Diego, CA, U.S.A.). The efficacy of the agonists was expressed as E_{max} (maximal response) and their potency as pEC_{50} ($-\log EC_{50}$, where EC_{50} is the concentration of the agonist required to produce half the maximal response). Since 5-HT activates different 5-HT receptor subtypes, the blocking potency of ketanserin (apparent pK_b) was estimated by

calculating concentration-ratios between the EC_{50} of agonist in the presence and absence of antagonist and plotting a Schild-plot, assuming a slope of unity. The antagonism of the response to sumatriptan by GR125743 only reached significance at concentrations of 100 nM or higher. Therefore, only two concentration points of antagonist were available, which prohibits the calculation of a Schild slope and pA_2 (concentration of the antagonist required to shift the concentration responses curve of agonist by two fold to right hand side). For that reason, the antagonist potency of GR125743 was also expressed as apparent pK_b, assuming a slope of unity. In case of UCA, the pK_b was calculated at 100 nM to allow a uniform comparison with data obtained with SFA, where only one concentration of the antagonists could be studied. The influence of the way of delivery (vaginal or caesarean section) on the response to KCl, 5-HT, sumatriptan or the antagonist was assessed by analysis of covariance (ANCOVA), using the way of delivery as a factor and gestational age as a covariant. Group means were compared by using unpaired Student t-test, with differences considered significant at $P < 0.05$. Correlation analyses were carried out using Pearson's coefficient of correlation between gestational age or neonatal weight and responses to 5-HT, ketanserin, sumatriptan or GR125743.

RESULTS

The demographic details of all the subjects from whom the umbilical cord artery, subcutaneous fat arteries, or both were obtained, are presented in Table 5.1. The KCl (100 mM)-induced contractions were not different between UCA obtained from normotensive and pre-eclamptic women. There were no differences between the responses to KCl, 5-HT, sumatriptan or the antagonists in the UCA obtained from vaginal deliveries and caesarean sections; therefore, the results were pooled in further analysis.

Table 5.1. Demographic details of the patients from whom the umbilical cord artery and subcutaneous fat arteries were obtained.

	Normotensive pre-term	Normotensive full-term	Pre-eclamptic pre-term	Pre-eclamptic full-term
Number	14	48	34	12
Maternal age (y)	30.6 ± 1.5	33.0 ± 0.7	32.9 ± 1.2	31.5 ± 1.6
Gestational age at delivery (wk)	30.6 ± 1.1	39.3 ± 0.1	30.2 ± 0.7	38.8 ± 0.4
Diastolic BP (mm Hg)	69.0 ± 0.9	74.7 ± 1.4	104.2 ± 2.5	98.2 ± 1.8
Systolic BP (mm Hg)	118.5 ± 0.9	121.1 ± 1.8	167.0 ± 5.9	144.8 ± 4.9
PCR (mg/mmol)	Not determined	Not determined	967 ± 293	699 ± 423
Birth weight (g)	1555 ± 193	3443 ± 73	1161 ± 99	3138 ± 131
Vaginal delivery	11	25	2	5
Caesarean section	3	23	32	7
Intra-uterine growth restriction	Not included	Not included	11	1

BP: Blood pressure, PCR: Protein/creatinine ratio

Within the pre-eclamptic group, patients with or without IUGR did not differ significantly in the responses to KCl, 5-HT, sumatriptan or their antagonists, hence their results were pooled in further analyses. In none of the UCA investigated, we observed an endothelium-dependent relaxation to substance P. The results obtained from UCA are presented in four main groups: the normotensive pre-term, the normotensive full-term delivery, the pre-eclamptic pre-term and the pre-eclamptic full-term delivery group.

Responses to 5-HT in umbilical cord artery

5-HT induced contractions in all UCA studied. The E_{max} and pEC_{50} of 5-HT were not significantly different between the groups (Figure 5.1, Table 5.2). Ketanserin antagonized the responses to 5-HT in a concentration-dependent manner in all groups with an average pK_b of around 7.6; there was no difference in the potency of ketanserin between the study groups. We did not observe any relaxations to 5-HT, putatively mediated by 5-HT_7 receptors, in precontracted UCA in the presence of ketanserin and GR125743 (both 100 nM, data not shown).

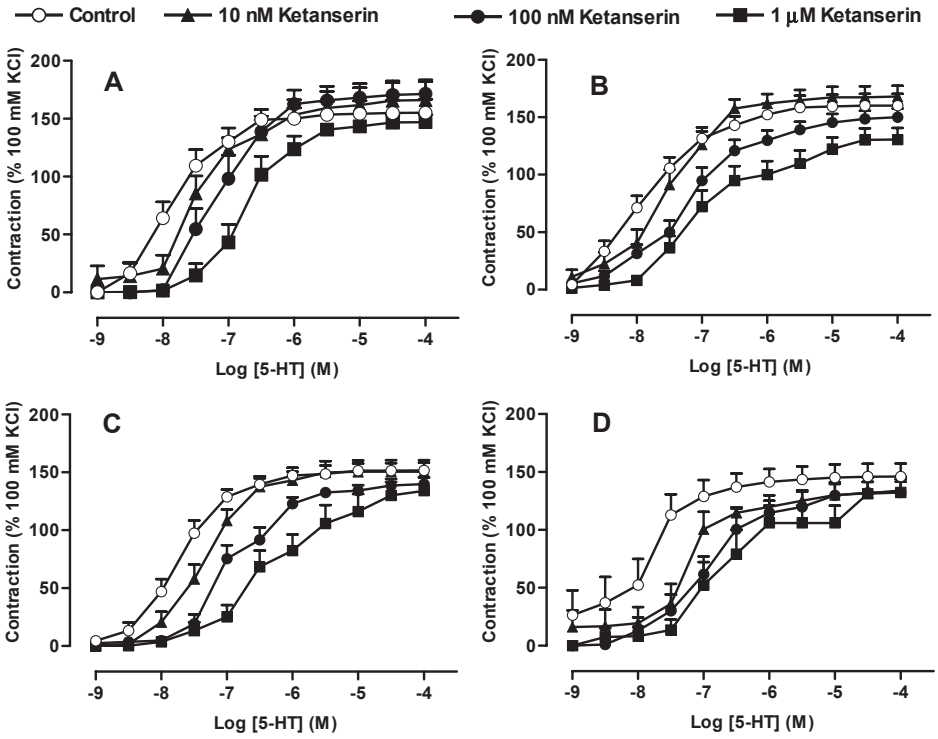

Figure 5.1. Concentration response curves to 5-HT in the absence or presence of increasing concentrations of the 5-HT_{2A} receptor antagonist, ketanserin (10 nM-1 μM), in umbilical cord artery segments. Panel A: normotensive pre-term, panel B: normotensive full-term, panel C: pre-eclamptic pre-term and panel D: pre-eclamptic full-term deliveries.

Responses to sumatriptan in umbilical cord artery

Sumatriptan also induced contractions in all UCA studied (Figure 5.2, Table 5.2). In the pre-term normotensive group, the pEC_{50} of sumatriptan (5.71 ± 0.23, n=12) was significantly lower than in the normotensive full-term group (6.62 ± 0.12, n=30). The E_{max} values between the groups were not significantly different. Within the pre-eclamptic group, there were no significant differences in E_{max} and potency of sumatriptan between pre-term and full-term in UCA. In the pre-eclamptic pre-term group, the potency of sumatriptan (6.26 ± 0.19, n=22) tended to be higher compared to the normotensive pre-term group, although this difference did not reach significance (P=0.08). GR125743 significantly antagonized contractile responses to sumatriptan at concentrations ≥ 100 nM, which is in accordance with previous observations in human coronary artery and saphenous vein [23]. Similar as observed with the potency of sumatriptan, the potency of GR125743 in the pre-eclamptic pre-term group (8.09 ± 0.22, n=17) tended to be higher compared to the normotensive pre-term group (P=0.06), while the potency of GR125743 was similar in the pre-eclamptic pre-term and pre-eclamptic

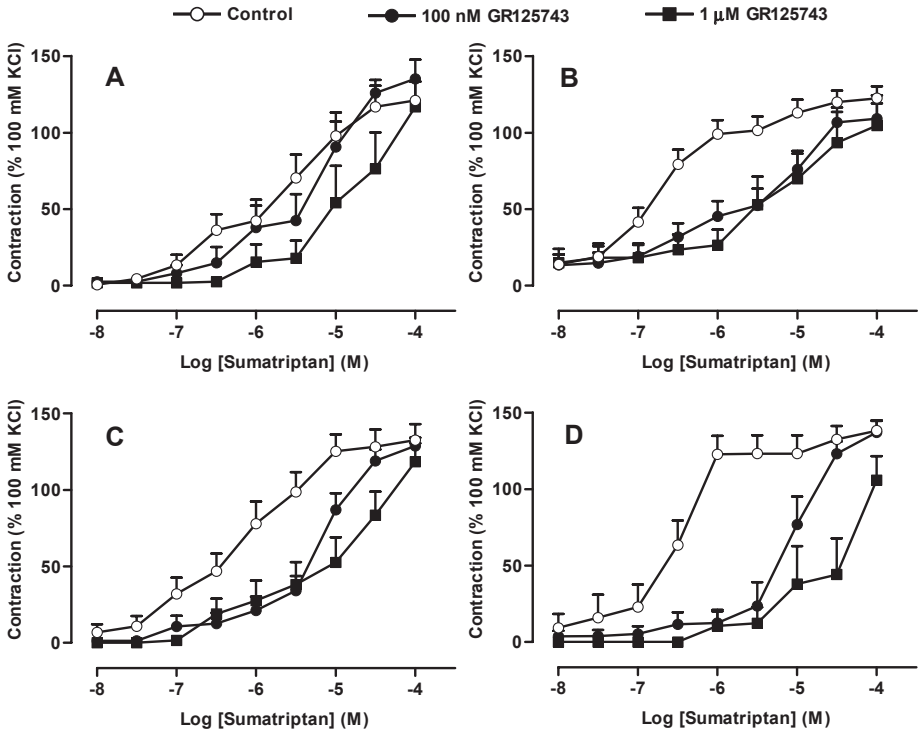

Figure 5.2. Concentration response curves to sumatriptan in the absence or presence of increasing concentrations of the 5-$HT_{1B/1D}$ receptor antagonist, GR125743 (10 nM-1 μM), in umbilical cord artery segments. Panel A: normotensive pre-term, panel B: normotensive full-term, panel C: pre-eclamptic pre-term and panel D: pre-eclamptic full-term deliveries.

Table 5.2. Pharmacological parameters derived from umbilical cord arteries obtained from normotensive pre-term and full-term pregnancies and pregnancies complicated by pre-term pre-eclampsia.

	Normotensive pre-term (n=7-14)		Normotensive full-term (n=18-39)		Pre-eclamptic pre-term (n=17-28)		Pre-eclamptic full-term (n=10-12)	
	5-HT	**Sumatriptan**	**5-HT**	**Sumatriptan**	**5-HT**	**Sumatriptan**	**5-HT**	**Sumatriptan**
E_{max} (% KCl)	155 ± 12	121 ± 12	160 ± 10	122 ± 8	152 ± 7	132 ± 10	146 ± 11	138 ± 7
pEC_{50}	7.89 ± 0.16	5.71 ± 0.23*	7.80 ± 0.14	6.62 ± 0.12	7.76 ± 0.11	6.26 ± 0.19	7.93 ± 0.19	6.48 ± 0.20
pK_b ketanserin	7.64 ± 0.24		7.77 ± 0.17	-	7.50 ± 0.13	-	7.67 ± 0.21	-
pK_b GR125743	-	7.28 ± 0.37*	-	8.05 ± 0.13	-	8.09 ± 0.22	-	8.19 ± 0.24
KCl (mN)	26.3 ± 3.1	26.3 ± 3.1	20.4 ± 1.6	20.4 ± 1.6	27.6 ± 2.5	27.6 ± 2.5	22.7 ± 2.8	22.7 ± 2.8

pK_b values of ketanserin and GR125743 determined at 100 nM; KCl: response to 100 mM KCl; n: number of UCA segments, each segment obtained from a different woman; *, Significantly different (P<0.05) from normotensive full-term.

Table 5.3. Pearson's coefficient of correlation between gestational age or neonatal weight and the agonist potency (pEC50) of 5-HT and sumatriptan as well as antagonist potency (pKb) of ketanserin and GR125743 to their respective agonists in umbilical cord artery segments obtained from normotensive controls (NT) and pre-eclamptic patients (PE).

Pearson coefficient	pEC50 5-HT		pKb Ketanserin		pEC50 Sumatriptan		pKb GR125743	
	NT	**PE**	**NT**	**PE**	**NT**	**PE**	**NT**	**PE**
Gestational age	-0.111	0.008	0.013	-0.092	0.456**	0.287	0.486*	0.146
Neonatal weight	-0.022	0.139	-0.028	-0.053	0.540***	0.260	0.424*	0.091
Number of patients	42	37	32	33	44	34	26	27

*P<0.05, **P<0.01 and ***P<0.001

full-term groups. In addition, the pK_b of GR125743 was significantly lower in normotensive pre-term deliveries (7.28 ± 0.37, n=7) compared to that in normotensive full-term deliveries (8.05 ± 0.13, n=18) in UCA, while such a difference was absent in UCA obtained from pre-eclamptic patients.

Relationship between foetal development and functional response to 5-HT, ketanserin, sumatriptan and GR125743 in umbilical cord artery

The fact that sumatriptan-induced responses were different in the UCA obtained from the pre-term and full-term normotensive deliveries with mean gestational ages of 31 and 39 weeks, respectively, suggests that 5-$HT_{1B/1D}$ receptors are still in a development phase during the third trimester. To study the effect of foetal development in further detail on the various pharmacological parameters obtained in the normotensive (both pre-term and full-term deliveries) and pre-eclamptic groups, we performed a correlation analysis with gestational age. We did not observe any significant correlation between the gestational age and the E_{max} or pEC_{50} of 5-HT or the pK_b of the 5-HT_{2A} receptor antagonist, ketanserin, in either group (Table 5.3). In contrast, there was a significant correlation between the gestational age and the potency of the 5-$HT_{1B/1D}$ receptor agonist, sumatriptan (r=0.456, P=0.002), as well as the potency of its antagonist, GR125743 (r=0.486, P=0.012), in UCA obtained from normotensive controls. In UCA obtained from patients suffering from pre-eclampsia, the potency of sumatriptan and its antagonist GR125743 did not correlate with gestational age. Since

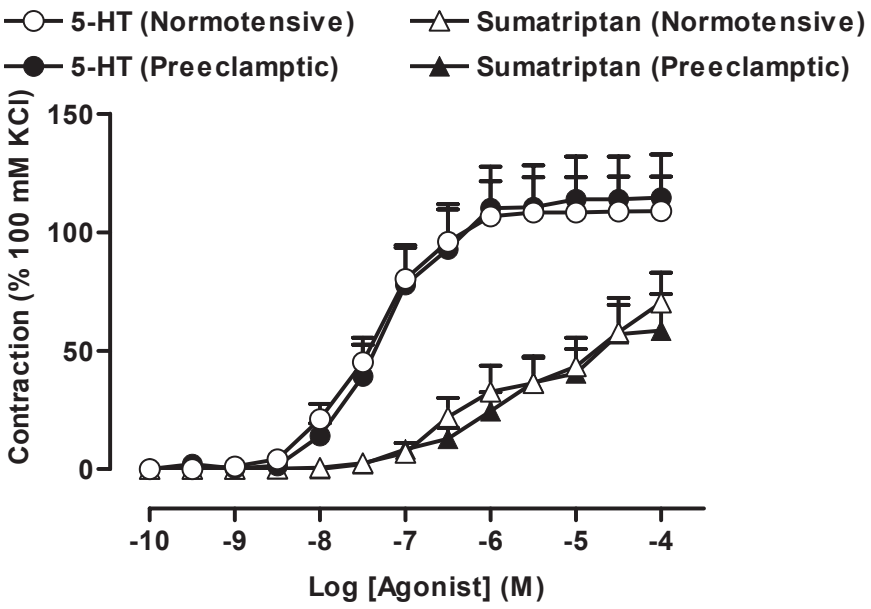

Figure 5.3. Concentration response curves to 5-HT and sumatriptan in subcutaneous fat resistance arteries obtained from normotensive and pre-eclamptic deliveries.

neonatal weight has been also described a marker for foetal development [24], we also analyzed the correlation between neonatal weight (that strongly correlated with gestational age, r=0.940, P<0.001) and the responses to the agonists and antagonists. In the pre-eclamptic group, twelve neonates with IUGR were present; we performed our correlation analysis either including or excluding these patients. As with the results obtained with gestational age, there was no significant correlation between the responses to 5-HT or ketanserin and neonatal weight in either of the groups. The potency of sumatriptan again significantly correlated with neonatal weight in the normotensive group (r = 0.540, P < 0.001), but not in the pre-eclamptic group (r = 0.260, P = 0.137 and r = 0.233, P = 0.262), including or excluding the patients with IUGR, respectively). There was a significant correlation between pK_b of GR125743 (r = 0.424, P = 0.031) and neonatal weight in the normotensive group, but not in the pre-eclamptic group (r = 0.091, P = 0.651 and r = 0.188, P = 0.415, including or excluding the patients with IUGR, respectively). Furthermore, there was a significant negative correlation between the gestational age and contractile responses to 100 mM KCl in UCA in normotensive group (r = 0.289, P = 0.044, n = 49), but not in the pre-eclamptic group (r = 0.211, P = 0.203, n = 38).

Responses to 5-HT and sumatriptan in subcutaneous fat arteries

One SFA in the normotensive group and three in the pre-eclamptic group did not respond to 5-HT or sumatriptan. These patients were not included in further analysis because the potency of the antagonists could not be assessed in these specimens. Because of the limited occurrence of deliveries by caesarean sections in pre-term normotensive patients, all the SFA from normotensive patients were analysed as one group, irrespective of their gestational age, unlike for UCA. There was no significant difference in the endothelium-dependent relaxations between SFA obtained from pre-eclamptic patients (46 ± 9% precontraction with U46619) and those obtained from normotensive women (37 ± 6%). Neither the maximal response or pEC_{50} of 5-HT, nor the potency of ketanserin was different between the pre-eclamptic and normotensive group (Table 5.4). Similarly, the responses to sumatriptan were not different between the two groups. In contrast, the $5\text{-HT}_{1B/1D}$ receptor antagonist, GR125743, was less potent in arteries obtained from pre-eclamptic patients than in normotensive controls.

Relationship between foetal development and responses to 5-HT, ketanserin, sumatriptan and GR125743 in subcutaneous fat arteries

The gestational age in the pre-eclamptic group (32.0 ± 1.2 weeks) was significantly less than that of the normotensive controls (38.9 ± 0.2 weeks). There was no significant correlation between gestational age or neonatal weight and responses to 5-HT or ketanserin in either of the groups. There was a significant correlation between the pEC_{50} of sumatriptan and neonatal weight, but not with gestational age, in the normotensive group. This correlation was absent in the SFA obtained from the pre-eclamptic patients. The potency of antagonist also did not have any significant correlation with neonatal weight or gestational age. The

correlation data should be interpreted with caution in view of the limited number of patients in the study group.

Table 5.4. Pharmacological parameters derived from subcutaneous fat arteries obtained from normotensive pregnancies and pregnancies complicated by pre-eclampsia.

	Normotensive (n=8-19)		Pre-eclamptic (n=8-16)	
	5-HT	**Sumatriptan**	**5-HT**	**Sumatriptan**
E_{max} (% 100 mM KCl)	109 ± 15	70 ± 13	115 ± 18	59 ± 15
pEC_{50}	7.18 ± 0.12	6.15 ± 0.26	7.39 ± 0.12	5.96 ± 0.25
pK_b Ketanserin (100 nM)	8.32 ± 0.36	-	8.43 ± 0.56	-
pK_b GR125743 (100 nM)	-	8.82 ± 0.24	-	8.01 ± 0.25*
Response to 100 mM KCl (mN)	4.72 ± 0.89	4.72 ± 0.89	6.46 ± 1.32	6.46 ± 1.32

n: number of UCA segments, each segment obtained from a different woman. *, Significantly different ($P<0.05$) from normotensive controls.

Comparison between subcutaneous fat arteries and umbilical cord arteries responses

In SFA, we observed endothelium-dependent relaxations, whereas in UCA these responses were absent. The maximal response and potency of 5-HT and sumatriptan were significantly lower in SFA than in UCA. In contrast, the potency of the antagonist ketanserin was higher in SFA than in UCA in both groups. In both UCA and SFA the maximal response to sumatriptan was less than the 5-HT response and in case of SFA, this difference was more pronounced.

DISCUSSION

In the current study, we investigated vasoreactivity in foetal and maternal arteries obtained from pre-eclamptic and normotensive pregnancies. As pregnancies complicated by pre-eclampsia are often associated with premature delivery, it is vital to take into account differences in gestational age while comparing with normotensive controls. Certain receptor systems are still in the developmental phase in the umbilicoplacental circulation in the third trimester of pregnancy. For example, in umbilical and placental veins, the reactivity of 5-HT and histamine tend to increase throughout this period [24]. Therefore, we included a correlation analysis between various pharmacological parameters obtained and markers of foetal development (gestational age and neonatal weight).

In UCA segments, obtained both from pre-eclamptic patients and normotensive controls, 5-HT and sumatriptan induced contractions that were sensitive to antagonism by ketanserin and GR125743, respectively. These results confirm the role of $5\text{-}HT_{2A}$ and $5\text{-}HT_{1B/1D}$ receptors in mediating vasoconstriction in UCA. Although we did not use selective $5\text{-}HT_{1B}$ or $5\text{-}HT_{1D}$

receptor antagonists, vasoconstriction to sumatriptan is most likely mediated via the 5-HT_{1B} receptor [25]. Even though 5-HT is not a selective 5-HT_{2A} receptor agonist, we chose 5-HT as an agonist in this study as it encompasses the study of all 5-HT receptor subtypes and provides the possibility to study the 5-HT_{2A} receptors using ketanserin. Further, the use of 5-HT allows comparison of our results with the other studies on 5-HT receptors in UCA [15,17,24,26]. We did not observe any difference in the 5-HT-induced contractions in UCA between the pre-eclamptic and the normotensive group, which seems to be in contrast to the finding by Bertrand *et al*. [24]. However, the higher E_{max} induced by 5-HT in UCA from pre-eclamptic patients in their study [24] is merely based on higher contractile responses to KCl, and disappears when the responses are corrected for the differences in absolute contractile force between the groups. Indeed, others also did not report any significant differences in the reactivity to various vasoconstrictors, including 5-HT, in UCA obtained from normotensive and pre-eclamptic patients [28] and some studies have even reported decreased responses to 5-HT in UCA obtained from severe pre-eclamptic patients [27]. In accordance with previous reports [24], we did also not observe any correlation between foetal development and the potency of 5-HT or ketanserin in UCA obtained from normotensive and pre-eclamptic patients. In conclusion, our findings suggest that 5-HT_{2A} receptors are not affected in pre-eclampsia, and that these receptors are already fully developed early in the third trimester of pregnancy.

In the study by Lovren *et al*. [17], a sub-threshold concentration of a vasoconstricting agent was required for observing the activity of 5-$HT_{1B/1D}$ receptors (only three out of ten vessels responded to sumatriptan without prestimulation), whereas in our study sumatriptan produced contractile response in all UCA without prestimulation. This discrepancy might be explained by higher levels of endogenous vasoconstrictors such as thromboxane A_2 in our preparations, which could then 'unmask' responses to sumatriptan [29,30] or could eventually be due to the higher pretension (25 mN) used in our study as used (20 mN) in the study by Lovren *et al*.

In our study, the potency of sumatriptan in UCA was about 10-fold higher in the full-term deliveries as compared to pre-term deliveries within the normotensive group, suggesting that 5-$HT_{1B/1D}$ receptors are still developing in the last trimester. This observation was strengthened by the significant positive correlation between foetal development (expressed as either gestational age or neonatal weight) and the pEC_{50} of sumatriptan in the normotensive group, whereas this correlation was absent in pre-eclamptic group. Similarly, the pK_b value of GR125743 only correlated with foetal development in the normotensive controls and not in the pre-eclamptic group. Additionally, unlike in the normotensive group, in the pre-eclamptic group the potency of sumatriptan does not seem to increase in full-term pregnancies, compared to the pre-term group. Finally, the pEC_{50} of sumatriptan and the pK_b of GR125743 in the normotensive pre-term control group appear to be lower than that in pre-eclamptic pre-term group, although this differences did not reach significance ($P = 0.06 - 0.08$). These observations suggest that in the pre-eclamptic group the normal

development of 5-$HT_{1B/1D}$ receptors is compromised, and that the response to these receptors may already be on higher strata at an earlier gestational period. This higher sensitivity may be explained by a larger number of 5-$HT_{1B/1D}$ receptors and/or a more efficient coupling of these receptors with the second messenger pathway in UCA of pre-eclamptic patients. The cause of this higher sensitivity could be increased plasma levels of 5-HT in pre-eclampsia[15], or, alternatively, foetal factors induced by umbilicoplacental vasoconstriction, which may contribute to maternal hypertension. Future studies, like binding experiments of 5-$HT_{1B/1D}$ receptors in foetal and maternal blood vessels at different gestational ages may give more insight into the role of these receptors in foetal development and pre-eclampsia.

A more prominent role of 5-$HT_{1B/1D}$ receptors in pre-eclampsia seems to gain relevance because contractile responses mediated by 5-$HT_{1B/1D}$ receptors may be augmented by other vasoconstrictive agents like thromboxane A_2 [26-29], and indeed plasma levels of both 5-HT and thromboxane A_2 are known to be increased in pre-eclampsia [4]. Interestingly, pre-eclampsia usually is manifested clinically in the third trimester, which is the same period where we observed an increase in the activity of 5-$HT_{1B/1D}$ receptors, suggesting that these receptors might have a pathophysiological role in pre-eclampsia. Antagonism of 5-HT receptors has been explored earlier as a therapeutic option for the treatment of pre-eclampsia using the 5-HT_{2A} receptor antagonist ketanserin [31,32], but in a substantial number of patients the antihypertensive response is insufficient [33]. However, the combination of ketanserin and aspirin, inhibiting the synthesis of thromboxane A_2, is beneficial in the prevention of pre-eclampsia in women with mild to moderate hypertension [31]. Since the augmentation of contractile responses to 5-HT by thromboxane A_2 is mainly mediated by 5-$HT_{1B/1D}$ and not by 5-HT_{2A} receptors [34], 5-$HT_{1B/1D}$ receptor antagonism might have an additive therapeutic value in the treatment of pre-eclampsia, especially by increasing the already compromised umbilicoplacental blood flow.

In SFA we did not observe any difference in substance P induced-relaxations between pre-eclamptic patients and normotensive controls, which seems to be at variance with earlier observations [35]. However, in a recent study performed on subcutaneous and myometrial resistance arteries, relaxations to substance P were not different between normotensive and pre-eclamptic subjects [12], which is in accordance with our observations. In UCA, we did not observe any relaxations to substance P, which is also in line with previous reports [36]. Both 5-HT and sumatriptan elicited contractions that were sensitive to antagonism by ketanserin or GR125743, respectively. Thus, our results demonstrate the presence of 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors in SFA. There were no significant differences in the reactivity to 5-HT or sumatriptan between the normotensive and the pre-eclamptic group. It should be noted that although vascular hyperreactivity has been described in pre-eclampsia [5], increased vascular sensitivity is not a generalized phenomenon in this syndrome, as we pointed out above [12,27,37] and as observed in the present study. Contractile responses to 5-HT and sumatriptan in SFA were significantly smaller than the responses observed in UCA, underscoring that humoral factors

play a more dominant role in contraction of umbilical cord arteries, which are not innervated. As in most studies in patients, pre-eclamptic patients were treated with antihypertensive drugs, which might induce an underestimation of differences between the pre-eclamptic and the normotensive pregnant women in our study.

Admittedly, our data suggest that antagonism of 5-$HT_{1B/1D}$ receptors would have a more prominent effect on the umbilicoplacental circulation than on maternal hypertension. However, since the umbilicoplacental blood flow tends to decrease after antihypertensive treatment of the mother [38], resulting in foetal distress, and factors released in response to a reduced blood flow to the foetus may also contribute to maternal hypertension, it is pivotal to maintain a sufficiently high umbilicoplacental blood flow.

In conclusion, we demonstrate that 5-$HT_{1B/1D}$ and 5-HT_{2A} receptors mediate contraction in foetal (UCA) and maternal (SFA) arteries. The functional profile of 5-HT_{2A} receptors does not change during the third trimester, nor is it different between pre-eclamptic pregnancies and normotensive controls. In contrast, the sensitivity of 5-$HT_{1B/1D}$ receptors increases during the third trimester in UCA in normotensive pregnancies, while this development is expedited in pre-eclamptic patients. Further studies on the role of 5-$HT_{1B/1D}$ receptors may give more insight into the role of 5-HT in foetal development and the pathophysiology of pre-eclampsia.

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Chapter 6

Ketanserin in pre-eclamptic patients; Transplacental transmission, transfer in breast milk and disposition in neonates

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ABSTRACT

The aim of this prospective, observational study was to assess the transplacental transmission and the transfer into breast milk after maternal use of ketanserin and to determine disposition in the neonate. In 22 pregnant women with severe pre-eclampsia, admitted to the antenatal ward in period 1999-2001, the ratio of drug concentrations in the umbilical cord to drug concentrations in maternal blood just before delivery was used as a indicator of placental transmission. Transfer into breast milk was determined by the ratio of the drug concentrations in breast milk as compared to the corresponding maternal plasma concentrations in seven patients. Disposition of ketanserin was assessed using neonatal plasma concentrations of ketanserin in eight neonates after birth.

A median placental transmission was found in the pre-eclamptic women of 0.95 (0.612-1.24) for ketanserin and for its metabolite, ketanserinol of 0.60 (0.5-0.77). The median breast milk/ plasma ratio for ketanserin was 0.4 (range, 0.2 – 1.27) and for ketanserinol 0.76 (range, 0.42 – 1.44). Pharmacologically relevant concentrations of ketanserin were found in the neonate after delivery. The elimination half-life of ketanserin in the neonate varied between 12.7 and 43.7 hours (median 19.3 hours) and of ketanserinol between 13.8 and 34.4 hours (median 18.7 hours). Despite the high placental transmission and disposition in the neonate, no apparent adverse effects in the neonates could be detected

In conclusion, a high placental transmission of both ketanserin and its metabolite ketanserinol and an intermediate transfer into breast milk occurred after maternal treatment of pre-eclampsia with ketanserin. Pharmacologically active concentrations of ketanserin were found in the neonate for a prolonged period after delivery.

INTRODUCTION

The mainstay of treatment of pre-eclampsia is to lower the maternal blood pressure by using (parenteral) antihypertensive drugs, in order to prevent the complications of the high blood pressure in the mother and, in selected cases, to prolong the pregnancy. The optimal antihypertensive drug for treatment of pre-eclampsia should establish a fast and controlled decline in maternal blood pressure, while having no adverse effects on mother, foetus or child.

Recently, the antihypertensive drug ketanserin, has been marketed in the Netherlands for treatment of pre-eclampsia. Ketanserin is a 5-HT_{2A} receptor antagonist with weak alpha-1-agonistic properties [1]. Presently, different views exist on the efficacy of ketanserin in severe pre-eclampsia[2], but the drug has the advantage of having limited adverse maternal effects [3] and it is relatively easy to administer. However, to assess the safety of maternal use of ketanserin for the foetus and neonate, information regarding transplacental transfer is important. Further, considering the long elimination half-life of ketanserin of 14h [4], data on the transfer into breast milk during and after ending of ketanserin treatment, are warranted, in order to advise mothers with regard to lactation. In humans, no quantitative data on placental transfer of ketanserin and its effect on the foetus are yet available, but animal studies in the pregnant ewe have demonstrated placental transfer of ketanserin [5]. Also, no data on transfer in breast milk are available.

In our university hospital, patients with severe, early onset pre-eclampsia are treated with high doses of ketanserin for prolonged periods. To establish whether adverse effects of ketanserin in the foetus or neonate can be demonstrated, we determined the degree of placental transfer of ketanserin and its metabolite, ketanserinol, as well as the degree of transfer into breast milk. Furthermore, the extent of exposure to ketanserin was studied in the neonate and the clinical outcome of the neonates was assessed.

METHODS

Patients with severe pre-eclampsia were started on ketanserin infusion upon admission to the antenatal ward in the period 1999 - 2001. Severe pre-eclampsia was defined in our study as the occurrence after 20 weeks of gestation of a diastolic blood pressure ≥ 110 mmHg and proteinuria ≥ 0.3 g/l or a diastolic blood pressure > 90 mmHg in combination with HELLP (haemolysis, elevated liver-enzymes, low platelet-count) syndrome. Ketanserin infusion was initiated at 4 mg/h and titrated, according to the blood pressure in increments of 2 mg/h every 20 minutes to a maximum of 20 mg/h. Each increment was preceded by an intravenous loading bolus injection of 5 or 10 mg ketanserin. Treatment was targeted at an intra-arterial diastolic blood pressure of ≤ 90 mmHg (Korotkoff sound 5, sphygmomanometer). Patients

who were already taking oral antihypertensive drugs on admission, such as methyldopa and/or nifedipine, continued using these medications.

Patients were treated with ketanserin until maternal or foetal conditions deteriorated and delivery was necessary. The study received permission from the Institutional Review Board of the Erasmus MC and all patients gave informed consent.

To determine placental transmission of ketanserin, a maternal plasma sample was obtained just before delivery and an umbilical cord blood sample was drawn after delivery. The plasma concentrations of ketanserin and ketanserinol were assessed using a validated reversed-phase high performance liquid chromatographic assay with fluorescence detection, which was developed at the hospital pharmacy [6]. The limit of quantification (LOQ) for both ketanserin and ketanserinol was 2.0 ng/ml. For analysis of ketanserin and ketanserinol in breast milk, the assay was again validated and the limit of quantification (LOQ) was 10 ng/ml for both compounds.

To determine the elimination of ketanserin in the neonate, two separate plasma samples were collected from the neonate within 36 hours after birth to measure the half-life of ketanserin. The elimination half-life ($t_{½}$) of ketanserin was calculated using the formula $t_{½} = 0.693\ (t_2\text{-}t_1) / \ln(conc_1) - \ln(conc_2)$.

Data analysis

The concentration ratios of ketanserin and ketanserinol of the umbilical cord blood (F) versus maternal plasma (M) were used as a measure of the placental transmission (F/M). For determination of the breast milk/plasma ratio, the concentration ratios of ketanserin and ketanserinol in breast milk versus the maternal plasma concentration of a simultaneously drawn plasma sample of the mother, were used.

Using the Spearman's correlation test ($p < 0.05$), we tested whether cumulative maternal dosage, duration of therapy or infusion rate before delivery, respectively, bore a significant correlation with the umbilical cord concentrations of ketanserin.

RESULTS

Twenty-two patients with 23 neonates were included (including one set of twins). The clinical characteristics of the patients and neonates are summarised in Table 6.1. One patient received ketanserin orally instead of intravenously. All babies were delivered by caesarean section. Ketanserin and ketanserinol could be detected in each of 44 maternal (ketanserin conc. 32 - 489 ng/ml, ketanserinol conc. 33 – 933 ng/ml) and umbilical cord plasma samples (ketanserin conc. 26 - 373 ng/ml, ketanserinol conc. 12 - 472 ng/ml).

Table 6.1. Maternal (n=22) and neonatal (n=23) clinical characteristics

Maternal characteristics	
Maternal age (years)	31.6 [20-40]
Gestational age at admission (weeks)	28 [18^{0}-31^{3}]
Gestational age at delivery (weeks)	30^{3} [26^{6}-34^{1}]
Nulliparity (n)	14 (64%)
Systolic blood pressure at admission (mm Hg)	175 [140-240]
Diastolic blood pressure at admission (mm Hg)	110 [95-120]
HELLP at admission (n)	18 (82%)
Indication for delivery:	
- foetal 'distress' (n)	13 (59%)
- maternal 'distress' (n)	6 (27%)
- combination foetal/maternal 'distress' (n)	2 (9%)
- spontaneous onset of labor (n)	1 (4.5%)
Neonatal outcome	
Ph umbilical cord	7.25 [7.00-7.38]
Apgar < 7 at 5 min (n)	4 (17%)
Birthweight (g)	1250 [520-2605]
Admission	
- Intensive Care Unit (n)	21 (93%)
- Medium or transitional Care (n)	1 (4%)
- No hospital admission (n)	1 (4%)
Mortality during admission (n)	3 (13%)
ICU stay (days)	6 [0-165]
Intraventricular hemorrhage grade III-IV (n)	2 (9%)
Artificial ventilation (n)	12 (53%)
Duration of ventilation (days)	4 [1-165]

Values are expressed as n (%) or mean [range]

The median transplacental transmission for ketanserin, calculated as ratio umbilical cord blood/maternal blood (F/M) was 0.95 (interquartiles 0.61 - 1.24, range 0.36 - 2.62). The mean placental transmission for ketanserinol was 0.60 (interquartiles 0.5 - 0.77, range 0.22 - 1.04). We found significant correlations between infusion rate before delivery (median 10 mg/h, range 2 - 18 mg/h) and umbilical cord concentrations ($r_s = 0.66$, $p < 0.05$), between duration of therapy (median 71 hours, range 3 - 895 hours) and umbilical cord concentrations ($r_s = 0.69$, $p < 0.01$) and between cumulative dosages (median 1900 mg, range 39 - 10,240 mg) and umbilical cord concentrations ($r_s = 0.79$, $p < 0.01$) for ketanserin.

We determined breast milk/plasma ratios in seven patients. In two patients administration of ketanserin was already stopped, but maternal ketanserin and ketanserinol were still detectable. In five patients the breast milk/plasma ratios were determined while using an intravenous dosage of ketanserin, ranging from 4 to 6 mg/h. We found ketanserin concentrations in breast milk ranging from < 10 (below LOQ) to 74 ng/ml and ketanserinol

concentrations ranging from 21 to 458 ng/ml. The median breast milk/plasma ratios were 0.4 (range, 0.2 – 1.27) for ketanserin and 0.76 (range, 0.42-1.44) for ketanserinol, respectively.

We were able to obtain two separate plasma samples from eight neonates (gestational age 26 6/7 - 32 4/7 weeks, birth-weight 670-1755 g) to determine the elimination half-life of ketanserin and ketanserinol. Maternal ketanserin use varied for these neonates between 2 - 12 mg/h during delivery and cumulative antenatal maternal ketanserin dosage ranged between 49 mg – 3295 mg. The individual plasma concentrations in each neonate are graphically depicted in Figure 6.1. The calculated half-life of ketanserin in these neonates varied between 12.7 and 43.7 hours (median 19.3 hours) and of ketanserinol between 13.8 and 34.4 hours (median 18.6 hours).

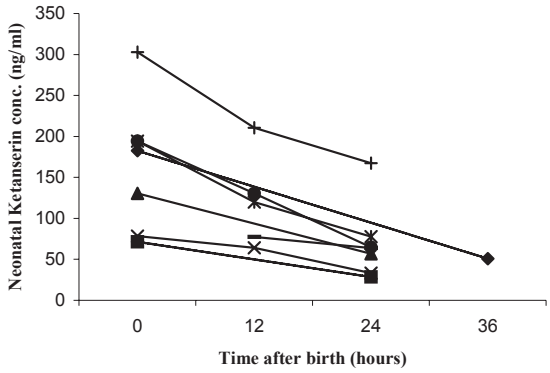

Figure 6.1 Neonatal plasma levels of ketanserin within 36 hours after birth, after maternal ketanserin use (n = 8).

DISCUSSION

Adequate information regarding exposure and possible effects of ketanserin on the human foetus and neonate in humans is lacking. Our study shows that maternal use of ketanserin results in high blood concentrations of ketanserin and its metabolite, ketanserinol, in the umbilical cord and in the neonate, indicating that ketanserin passes the placenta easily.

The transplacental passage of ketanserin is in accordance with our expectations, considering its physicochemical properties (moderate lipophilic, molecular weight of 365 Da, pK_a = 7.50) [4]. Being a weak base, ketanserin is highly un-ionised at the physiologic blood pH of the mother, which facilitates diffusion through the placenta. After passing the placenta, the slightly more acidic pH of the foetus might result in a shift of un-ionised ketanserin to a higher fraction of ionised ketanserin, which makes redistribution through the placenta back to the mother more difficult (ion-trapping).

In the mothers, ketanserin is metabolised in the liver to ketanserinol [4]. The affinity of the latter compound for the 5-HT_{2A} receptor is about a thousand- fold less compared to the parent drug ketanserin. However, a reduction-oxidation equilibrium seems to exist between ketanserinol and ketanserin, resulting in an indirect pharmacological effect of ketanserinol. Ketanserinol is more hydrophilic than ketanserin, explaining the lesser degree of placental transmission for ketanserinol, compared with ketanserin that we found in this study. We made the assumption that the ketanserinol-levels found in the umbilical cord were of maternal origin rather than metabolised by the foetus because, although not specified in the protocol, usually venous umbilical cord blood was taken, reflecting the blood flow from mother to the foetus. Also, the activity of most liver-enzymes in the foetus is low, thus the foetal contribution to the formation of ketanserinol is likely to be marginal.

We found a correlation between ketanserin levels in the umbilical cord and cumulative maternal dosage, duration of therapy and dosage before delivery, respectively. This correlation was in accordance with our expectations, as diffusion of the drug which is both concentration and time dependent, will play an important part in the placental transfer. The F/M ratio, used as a measure of placental transmission, varied extensively in our study. This variation can probably be explained not only by differences in maternal exposure to ketanserin, but also by other individual factors, which will influence placental transfer, such as maternal plasma protein concentration and placental blood flow.

The concentrations of ketanserin determined in the umbilical cord plasma samples and in the neonate are comparable to and sometimes even higher than, the therapeutic plasma concentrations in adult patients treated with ketanserin for chronic hypertension (15-140 ng/ml) [7]. This implies that pharmacological effects in the foetus and neonate are to be expected. Bolte et al [8] reported placental transmission of ketanserin, but did not detect any apparent adverse effects in the neonate that could be attributed to the drug. Rossouw [9] confirmed the apparent safety of ketanserin for mother and foetus, but dosages were lower than used in our study. In our study, no apparent side effects were found in foetus or neonate, but because the study was non-comparative, no valid conclusions can be drawn. For other antihypertensive drugs, used in pregnancy, e.g. labetalol, methyldopa, nifedipine and hydralazin, extensive placental transfer has also been described [10].

The breast milk/plasma ratios found in our study, indicate that the neonate can be exposed to substantial amounts of ketanserin and ketanserinol during lactation. These findings support our current policy of discouraging nursing of the neonate while maternal ketanserin concentrations are still expected to be in the pharmacologically active range.

In our study, the elimination half-life of ketanserin was prolonged in the neonate, as compared with the elimination half-life of 14.3 hours (± 4.4), found in adult volunteers[4]. Neonates apparently clear ketanserin at a slower rate as compared to adults, probably caused by immature metabolising systems in the liver of the neonate, especially in small for gestational age or premature neonates, as seen often in pre-eclamptic pregnancies. For the

assessment of the elimination rate, we have included in our study only neonates who stayed in our hospital for at least two days, for logistical purposes. This might have introduced a bias, since these babies probably are the most ill. However, the majority of the neonates, 19 of 23 babies stayed at the hospital for a longer period. The neonates studied, spanned a wide range of gestational age, birth weight and amount of maternal drug use, and so this study population is representative of newborns from patients with early-onset pre-eclampsia. Long term effects of foetal exposure to ketanserin were not studied but other workers [11] have not found effects in infants up to six years after exposure.

CONCLUSION

A high placental transmission and an intermediate transfer into breast milk of ketanserin and its metabolite ketanserinol occurred after maternal treatment of pre-eclampsia with ketanserin. Pharmacologically relevant concentrations of ketanserin were found in the neonate for a prolonged period after delivery.

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Chapter 7

The effect of maternal ketanserin treatment on foetal 5-HT receptor function in umbilical cord artery of pre-eclamptic patients

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ABSTRACT

Maternal treatment with the 5-HT_{2A} receptor antagonist ketanserin (KT) in pre-eclamptic patients is associated with a high placental transmission of KT, resulting in pharmacologically active levels of KT in umbilical cord artery (UCA) and neonate. To study whether exposure to KT influences the characteristics of foetal 5-HT receptors, functional studies were performed on 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors in UCA from pre-eclamptic patients treated with KT.

UCA were obtained, immediately after delivery, from pre-eclamptic patients (n=7), treated antenatally with intravenous ketanserin. Pre-eclamptic patients (n=13), not treated with KT (non-KT), were included as a control group. Segments of UCA were prepared and mounted in tissue baths and isometric force changes were determined. Cumulative concentration-response curves to 5-HT and to the 5-$HT_{1B/1D}$ receptor agonist sumatriptan, were constructed in the absence or presence of the 5-HT_{2A} receptor antagonist ketanserin or the 5-$HT_{1B/1D}$ receptor antagonist GR125743, respectively.

All UCA segments showed contractile responses to both 5-HT and sumatriptan, and the concentration-response curves showed a rightward shift with increasing concentrations of KT and GR125743, respectively, indicating the presence of functional 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors in the foetal tissue. No significant differences were found in maximum response E_{max} (expressed in % of response on 100 mM KCl), or potency (pEC_{50}) of 5-HT in both groups (E_{max} 142 ± 11.6 %, pEC_{50} 7.64 ± 0.26 in KT-treated group and E_{max} 162 ± 12.6 %, pEC_{50} 7.69 ± 0.14 in non-KT treated group, respectively). No significant differences were found in the potency of the antagonist ketanserin in both study groups (pK_b 7.58 ± 0.37 in KT group and 7.46 ± 0.17 in non-KT group, respectively). Similarly, with sumatriptan, no significant differences were found between KT-treated patients and non-KT treated patients (E_{max} 132 ± 20.3 % and 140 ± 14.7%, respectively, pEC_{50} 5.98 ± 0.29 and 6.41 ± 0.28 respectively, pK_b of GR125743 7.53 ± 0.27 and 8.43 ± 0.29, respectively).

In conclusion, foetal exposure to ketanserin in pre-eclamptic patients does not significantly influence the functional characteristics of 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors in the umbilical cord artery.

INTRODUCTION

Pre-eclampsia is a disease occurring in 2-5% of the pregnant women and it forms one of the leading causes of maternal and neonatal mortality and morbidity during pregnancy [1]. The pathophysiology of the disease is not yet fully understood, but impaired trophoblast invasion and endothelial dysfunction are considered to be important factors in the pathogenesis[1,2]. The main clinical characteristics of pre-eclamptic patients are elevated blood pressure, proteinuria and oedema, occurring after the twentieth week of gestation. Treatment with antihypertensive drugs is indicated to stabilise the patient and prevent maternal complications like organ failure or haemorrhages in retina or brain. Preterm delivery of, often severely growth restricted, neonates occurs frequently in early-onset pre-eclamptic patients [3,4].

In the past years, prolonging the pregnancy of early-onset pre-eclamptic patients ("temporising management"), using potent antihypertensive drugs, has been undertaken to improve neonatal outcome [5-7]. One of the antihypertensive drugs used most often in The Netherlands in the treatment of pre-eclampsia, is the 5-HT_{2A} receptor antagonist ketanserin (KT). The drug is thought to act by blocking the vasoconstrictive response to 5-HT in the blood vessels [8]. An increased vasoconstrictive response to 5-HT, leading to increased peripheral resistance, has been implicated as one of the mechanisms involved in pre-eclampsia. Hence, the use of a 5-HT antagonist such as KT, is considered a rational approach in the treatment of pre-eclampsia [9,10]. However, for temporising management in severe, early-onset pre-eclamptic patients, high intravenous dosages of KT are needed for prolonged periods of time, resulting in extensive foetal exposure to KT. Indeed, concentrations of KT equal to maternal levels, were found in the umbilical cord (26 - 373 ng/ml) and in the neonate (71 - 302 ng/ml) after maternal use of KT [11]. Whether these pharmacologically active concentrations of ketanserin, being a 5-HT_{2A} receptor antagonist, influence the characteristics and possibly cause desensitisation of foetal 5-HT receptors, is unknown. In animal studies, chronic blockade of 5-HT_{2A} receptors by KT has been shown to lead to an unexpected down-regulation of 5-HT_{2A} receptors [12, 13].

5-HT is known to be one of the earliest neurotransmitters produced during foetal brain development [14, 15]. It can be speculated that foetal exposure to 5-HT receptor blocking drugs such as ketanserin, can cause harmful effects on the foetus, especially considering the abundant presence of 5-HT receptors in the foetal brain. Although human studies have not yet shown clinical adverse effects in neonates, clearly attributable to maternal KT treatment [8, 11, 16] studying foetal 5-HT receptors in functional studies will yield more detailed information regarding foetal development of these receptors and may lead to a better assessment of the risk of long-term effects in the neonate after maternal KT treatment. In our study, we selected the umbilical cord artery as representative of foetal vessels, knowing that 5-HT_{2A} and 5-$HT_{1B/1D}$ are the major receptors mediating 5-HT induced vasoconstriction in human umbilical cord artery [17].

Based on the results of animal studies [12,13], we hypothesise that prolonged exposure of tissue and blood vessels to the 5-HT_{2A} receptor antagonist ketanserin influences the functionality of foetal 5-HT receptors, by downregulation of the 5-HT_{2A} receptor.

In the present study, functional responses of 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors in UCA from pre-eclamptic patients treated with ketanserin were compared with responses from pre-eclamptic patients not treated with KT.

METHODS

Twenty pre-eclamptic patients, admitted to the antenatal ward of the Erasmus MC were included in the study in the period 2002 - 2004. The Ethics Committee of the Erasmus MC approved the protocol and all patients gave informed consent prior to inclusion.

Pre-eclampsia was defined as the occurrence, after 20 weeks of gestation, of a diastolic blood pressure ≥ 110 mmHg and a protein/creatinin ratio ≥ 30 mg/mmol creatinin, or the occurrence of a repetitive diastolic blood pressure ≥ 90 mmHg in combination with the HELLP (haemolysis, elevated liver-enzymes, low platelet-count) syndrome.

Patients were divided into two study groups; one study group was treated with KT before delivery and the other study group treated with other antihypertensive drugs (dihydralazin, nifedipine or nicardipine) without exposure to KT. All patients in the KT group and the majority of patients (85%) in the non-KT group used methyldopa orally.

KT treatment consisted of an intravenous bolus injection of 10 mg followed by a continuous infusion of KT at 4 mg/h. According to the blood pressure, the infusion rate of KT was increased with 2 mg/h every 20 min to a maximum of 20 mg/h. Each increment was preceded by an intravenous loading bolus injection of 10 mg KT. Drug treatment was targeted at an intra-arterial diastolic blood pressure of ≤ 90 mmHg. Antihypertensive treatment was continued as long as foetal and/or maternal condition did not warrant delivery, as judged by the attending obstetrician.

The umbilical cords were collected immediately after caesarean or vaginal deliveries. Umbilical cord was collected in Krebs solution at 4 °C (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.1, pH 7.4), transported to the laboratory and UCA was isolated from the umbilical cord. Functional experiments were performed on the same or subsequent day. Segments of UCA of 3-4 mm length and 1.5-2 mm internal diameter were suspended with help of stainless-steel hooks in 15 ml organ baths filled with carbogenated (95% O_2 / 5% CO_2) Krebs solution at 37 °C. Each segment was set under a tension of 25 mN, as determined to be the optimal tension in pilot experiments. The segments were washed after every 15 min and were allowed to equilibrate for 45 min, to ensure that no maternal ketanserin was present in the vessels before start of the experiments.

Two successive challenges to KCl (30 mM, Merck, Darmstad, Germany) were performed to check the reproducibility of the response. 100 mM KCl was subsequently added to determine the reference contractile response of the segment. Serotonin (5-HT, Sigma Chemicals Co., Steinheim, Germany) and sumatriptan (Pfizer Ltd, Sandwich Kent, U.K.) were added to different segments in a cumulative manner in the absence or presence of antagonists. Concentration response curves to 5-HT or to sumatriptan were constructed in a parallel set-up in presence of vehicle or after 30 min of incubation with the 5-HT_{2A} receptor antagonist ketanserin (10 nM, 100 nM or 1μM) (Pfizer Ltd, Sandwich Kent, U.K.) or the 5-$HT_{1B/D}$ receptor antagonist GR125743 (10 nM, 100 nM or 1 μM) (Pfizer Ltd, Sandwich Kent, U.K.) [18], respectively. All agonists and antagonists were dissolved in distilled water and stored in aliquots at -80 °C. Only a single concentration-response curve was constructed in each artery segment.

Data and statistical analysis

Clinical characteristics between the two groups were compared using Wilcoxon's rank sum test. All contractile responses to the agonists were expressed as percentage contraction of the tone induced by 100 mM KCl. All values were expressed as mean ± S.E.M. The concentration response curves for the agonists were analysed using non-linear regression analysis (Graph pad Prism 3.01, Graph pad Software Inc., San Diego, CA, U.S.A). The potency of agonist was expressed as pEC_{50} (-log(EC_{50})) and the blocking potency of the antagonists (pK_b) was estimated by calculating concentration-ratios between EC_{50}-values of agonist in the presence and in the absence of antagonists and plotting a Schild-plot [19], assuming a slope of unity.

Statistical analysis was performed using SPSS (version 11.5, SPSS Inc, Chicago, USA). Statistical significance was determined by the Students t-test, with differences considered significant at $p < 0.05$. A post hoc power analysis was performed to verify whether sufficient patients were investigated. Correlation coefficients between pEC_{50}, E_{max} or pK_b and duration of KT treatment, cumulative dosage or maximum dosage was calculated according to Pearson's coefficient of correlation (r).

RESULTS

The demographic and clinical characteristics of the seven KT- treated and thirteen non-KT treated patients included in this study, are summarized in Table 7.1. The groups did not differ significantly with respect to age, gestational age at admission or delivery, blood pressure at admission, neonatal weight or way of delivery. In all KT- treated patients, initially adequate blood pressure control was achieved with KT, but in two patients alternative intravenous antihypertensive drugs (nicardipine n=1, dihydralazin n=1) were added to maintain adequate blood pressure control. In one patient in the KT- treated group, KT treatment was stopped two hours before delivery and one patient stopped KT treatment 45 hours before delivery.

These patients were included in the analysis in the KT group because possible effects of maternal KT treatment on umbilical cord receptors were assumed to persist at least several days, considering the half-life of 5-HT_2 receptors of 3 to 5.5 days [20] and the elimination half-life of KT of 13 - 18 h [21].

Table 7.1. Clinical characteristics of pre-eclamptic patients, treated with ketanserin before delivery (n=7) and treated with other drugs (nifedipine, dihydralazin or nicardipine) before delivery (n=13). Data are expressed as median (range) or number (%).

	Pre-eclamptic patients treated with ketanserin (n=7)	Pre-eclamptic patients not treated with ketanserin (n=13)
Age (years)	34 (25-42)	31 (18-44)
BP diastolic at admission (mmHg)	105 (85[1]-120)	100 (90-120)
BP systolic at admission (mmHg)	180 (140-220)	160 (110-200)
Protein/creatinin ratio (mg/mmol)	355 (80-1856)	470 (127-1257)
Gestational age at admission (weeks)	28 (25 4/7 – 36 3/7)	29 4/7 (24 4/7 –34 6/7)
Gestational age at delivery (weeks)	31 (26 4/7 – 36 3/7)	30 6/7 (26 3/7 – 36 6/7)
Way of delivery		
- Caesarian (n)	7 (100%)	12 (92%)
- Vaginal (n)	0	1 (8%)
Twin gestation (n)	0	1 (8%)
Ketanserin use		
- Duration of treatment (h)	54 (6-399)	-
- Maximum dosage (mg/h)	9 (6-18)	-
- Cumulative dosage (mg)	379 (47-3055)	-
Neonatal weight (g)	1035 (750-2250)	1205 (650-2165)
- below 10th percentile (n)	2 (28%)	6 (46%)
- below 2.3th percentile (n)	1 (14%)	1 (8%)

[1] One patient with severe HELLP-syndrome

The response to 100 mM KCl did not differ significantly between the study groups in the KT-treated group (35 mN ± 16) versus the non-KT-treated group (24 mN ± 10).

5-HT and the 5-$HT_{1B/1D}$ agonist, sumatriptan, induced potent contractions in the UCA in both groups, which did not differ with respect to E_{max} and pEC_{50} (Figure 7.1, Table 7.2). The concentration- response curves to 5-HT showed a rightward shift after exposure to increasing concentrations of the 5-HT_{2A} antagonist ketanserin (Table 7.2, Figure 7.2a and 7.2b). Similarly, 30 min incubation with the 5-$HT_{1B/1D}$ antagonist, GR125743, resulted in a rightward shift of the concentration-response curves to sumatriptan (Table 7.2).

No statistical differences were found between the pK_b values of 5-HT receptors in UCA of pre-eclamptic patients treated with KT or those not treated with KT. Although our sample size was limited, a post hoc power analysis (α=0.05, β=0.8) showed that the group size was sufficiently large to detect a difference in pEC_{50} of 0.7, corresponding to a five-fold difference in potency, which we consider to be clinical relevant.

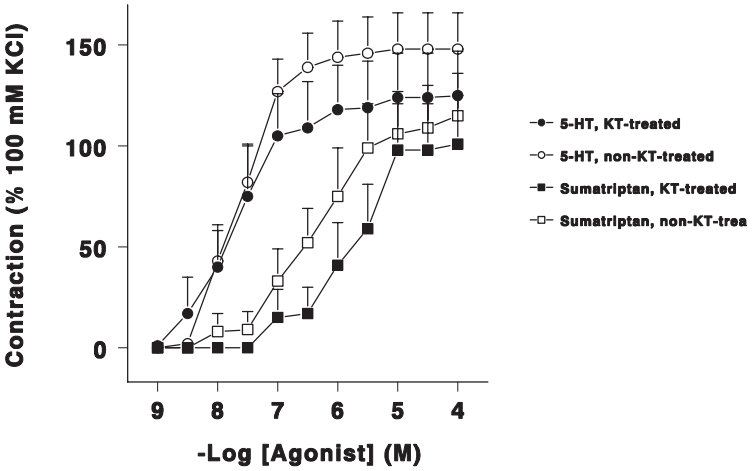

Figure 7.1 Concentration response curves to 5-HT in umbilical cord artery segments of ketanserin treated patients (●) (n = 7) and of patients, not treated with ketanserin (o) (n = 13) and concentration response curves to sumatriptan in umbilical cord artery segments of ketanserin treated patients (■) (n = 7) and of patients, not treated with ketanserin (□) (n = 13).

No correlations were found for 5-HT for E_{max} or pEC_{50}, with duration of KT treatment (for E_{max}, r = -0.573, p = 0.179; for $pEC_{50,}$ r = -0.252, p = 0.585), cumulative dosage (for E_{max}, r = -0.552, p = 0.199; for $pEC_{50,}$ r = -0.342, p = 0.454) and maximum dosage respectively (for E_{max}, r = -0.095, p = 0.842; for $pEC_{50,}$ r = -0.281, p = 0.542). Similarly, no correlations were found for sumatriptan for E_{max} or pEC_{50}, with duration of KT treatment (for E_{max}, r = 0.185, p = 0.691; for $pEC_{50,}$ r = 0.077, p = 0.869), cumulative dosage (for E_{max}, r = 0.228, p = 0.623; for $pEC_{50,}$ r = 0.162, p = 0.728) and maximum dosage respectively (for E_{max}, r = 0.656, p = 0.109; for $pEC_{50,}$ r = 0.052, p = 0.911).

Table 7.2. 5-HT receptor characteristics, expressed as mean (± S.E.M.) of Emax, pEC50 and pKb, from umbilical cord arteries obtained from pre-eclamptic patients treated with ketanserin and from pre-eclamptic patients not treated with ketanserin. 5-HT and sumatriptan were used as agonists in absence or presence of ketanserin (100 nM) and GR125743 (100 nM) as their respective antagonists.

	Preeclamptic patients treated with ketanserin		**Pre-eclamptic patients not treated with ketanserin**	
	5-HT	**Sumatriptan**	**5-HT**	**Sumatriptan**
E_{max} [1]	142 ± 11.6 (n=7)	132 ± 20.3 (n=7)	162 ±12.6 (n=11)	140 ± 14.7 (n=11)
pEC_{50}	7.64 ± 0.26 (n=7)	5.98 ± 0.29 (n=7)	7.69 ± 0.14 (n=11)	6.41 ± 0.28 (n=11)
pK_b	7.58 ± 0.37 (n=5)	7.53 ± 0.27 (n=4)	7.46 ± 0.17 (n=10)	8.43 ± 0.29 (n=8)

[1]expressed as percentage of the response induced by 100 mM KCl.

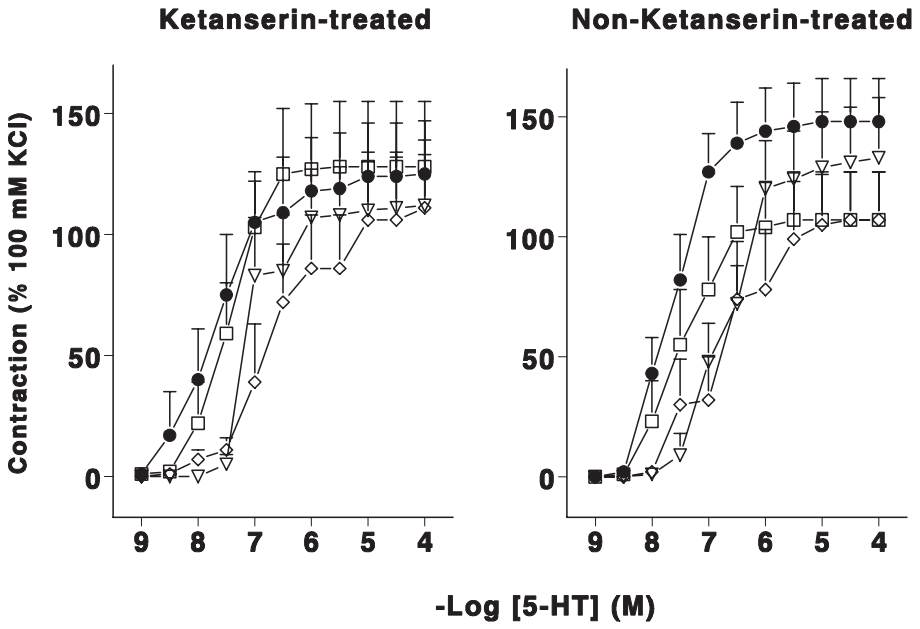

Figure 7.2 Concentration response curves to 5-HT in the absence (●) or presence of increasing concentrations of the 5-HT2A receptor antagonist, ketanserin (10 nM ( ), 100 nM (▽) and 1 μM (◊), in umbilical cord artery segments of ketanserin treated patients (n=7) and of patients, not treated with ketanserin (n=13).

DISCUSSION

The presence of 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors in utero-placental vessels of normotensive patients has been described by several authors [22-24]. Increased concentrations of 5-HT have been reported at birth in maternal and placental circulation [25], indicating a role for 5-HT in maintaining vascular tone at birth. In pre-eclampsia, Middelkoop et al [26] have shown an increase in maternal circulation of 5-HT in pre-eclamptic patients, suggesting a role for 5-HT in the aetiology of pre-eclampsia.

In agreement with these studies, we found that 5-HT and sumatriptan induced contractions in all UCA tested, indicating the presence of 5-HT receptors. Ketanserin and GR125743 moved the 5-HT and sumatriptan curves rightwards in concentration-dependent manner, confirming the presence of functional 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors in umbilical cord artery.

The pK_b values (7.58 and 7.46) of ketanserin against 5-HT are one log unit lower than the pA_2 values of 8.7 - 8.9 described by other authors for umbilical cord artery [17, 27]. This might be explained by the fact that the blocking activity of ketanserin is being underestimated in our experiments because 5-HT will have functional affinity for 5-HT_1 receptor subtypes as well.

Even though 5-HT is not a selective 5-HT_{2A} receptor agonist, we chose 5-HT as an agonist in this study as it encompasses the study of all 5-HT receptor subtypes and allows comparison of our results with other studies on 5-HT receptor functionality [17, 28, 29]. Furthermore, our results are in line with previously reported values of pA_2 of 7.7 - 7.85 for 5-HT_{2A} receptor in animal tissue [30, 31]. The pK_b value of GR125743 (7.53-8.43) for sumatriptan is in accordance with previously observed pA_2 values of 8.18 in human coronary artery and 8.34 in human saphenous vein [32].

The 5-HT_{2A} receptor antagonist KT has been used increasingly in recent years as an antihypertensive drug in pre-eclamptic patients. However, the substantial transplacental transmission and subsequently high foetal exposure to KT [11] may lead to adverse effects on foetal 5-HT receptors. The early appearance and continued expression of foetal 5-HT receptors during gestation has been demonstrated in animal studies [15]. Lauder et al [15] showed in mouse embryos that both structural as well as functional damage occurred after foetal exposure to high dosages of the 5-$HT_{2A/2C}$ receptor antagonist, mianserin and the 5-$HT_{2A/2B/2C}$ receptor antagonist, ritanserin. No malformations were found after foetal exposure to KT. Whitaker et al [33] demonstrated in animal studies that foetal exposure to the 5-HT-agonist, 5-methoxytryptamine, or exposure to a decreased level of maternal 5-HT (achieved by adding a tryptophan hydroxylase inhibitor antagonist) resulted in downregulation and upregulation of 5-HT receptors in new born offspring, respectively.

In humans, only data on foetal effects after use of selective serotonine re-uptake inhibitors (SSRI's) during pregnancy are available. These drugs have shown to affect foetal 5-HT regulation, resulting in adverse effects on neonatal behaviour (tremor, restlessness, rigidity postnatally) following gestational exposure [34, 35].

Based on the aforementioned data on adverse foetal effects after maternal drug use, we studied the effect of maternal KT treatment on foetal 5-HT receptor characteristics in pre-eclamptic patients. We hypothesized, based on animal studies [12, 13], that foetal exposure to KT might influence the functionality of 5-HT_{2A} receptor, but not of the 5-$HT_{1B/D}$ receptor.

Our data show no significant differences in 5-HT- or sumatriptan-induced vasoconstrictive responses of the UCA between the pre-eclamptic group treated with KT and the pre-eclamptic group without exposure to KT, indicating that exposure to KT does not influence foetal 5-HT_{2A} and 5-$HT_{1B/1D}$ receptor characteristics in UCA.

Dosage and duration of treatment

It can be expected that high dosages of KT and long-term treatment will exert a more pronounced effect on receptor characteristics than lower dosages, although all dosages used in this study were in the pharmacologically active range and have been known to cause high umbilical cord plasma levels of KT [11]. These plasma levels are in the same range as the concentrations KT used in our *ex vivo* experiments, indicating that results obtained with our experiments are of clinically relevance.

In our study no relationship between dosage and $pEC_{50,}$ E_{max} or pK_b could be established. It should be born in mind that the number of patients in our study was relatively small, due to the fact that many early-onset pre-eclamptic patients need alternative treatment before delivery after the maximum dosage of ketanserin is reached. However, power analysis established that statistical power in our study was sufficient to detect clinically relevant differences.

Gestational age

Limited information is available with respect to the influence of gestational age on the response of the umbilical artery to 5-HT. Bertrand et al [28] showed an increase in umbilical and placental veins sensitivity to serotonin throughout the third trimester in normotensive patients, whereas this change was not seen in pre-eclampsia. In our study the study groups did not differ with respect to gestational age, therefore a possible confounding factor of gestational age on the results was excluded. However, it can be speculated that clinical effects of maternal KT treatment on receptor population will (partly) depend on gestational age, if sensitivity to 5-HT in UCA indeed changes with gestational age.

Our results, suggesting a lack of effect of maternal KT treatment on functionality of 5-HT receptors in UCA support the currently available clinical data, showing that use of KT in pre-eclamptic patients is safe for foetus and neonate.

In conclusion, prolonged foetal exposure to ketanserin in pre-eclamptic patients does not seem to influence the functional characteristics of $5\text{-}HT_{2A}$ and $5\text{-}HT_{1B/1D}$ receptors in the umbilical cord artery.

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Chapter 8

Intravenous use of the calcium-channel blocker nicardipine as second-line treatment in severe, early-onset pre-eclamptic patients

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ABSTRACT

To evaluate the efficacy of intravenous administration of nicardipine as second-line temporising treatment in pre-eclamptic patients, a prospective study was performed in twenty-seven early-onset, pre-eclamptic patients with a median gestational age of 27 weeks (range 21^{2} – 32^{1} weeks), with treatment failure on standard intravenous antihypertensive drugs (ketanserin, dihydralazin or labetalol). Nicardipine infusion was started for temporising management of pre-eclampsia at a dosage of 3 mg/h and was subsequently titrated according to blood pressure. Nicardipine treatment was continued as long as maternal and foetal condition allowed. The endpoints of the study were defined as percentage of patients reaching the target diastolic intra-arterial bloodpressure (< 100 mmHg or < 90 mmHg in HELLP-syndrome patients) within 1 h after the start of treatment, and the number of days of prolongation of pregnancy under nicardipine treatment. Maternal and foetal side effects, foetal death and neonatal outcome were assessed.

In all patients target diastolic blood pressure was obtained within a median of 23 min (range 5 - 60 min). Delivery was postponed for a median of 4.7 days (range 1 - 26 days) using nicardipine treatment, in a maximum dosage ranging from 3 to 9 mg/h. Detailed haemodynamic parameters with corresponding nicardipine dosages were obtained in nine patients. In 30% of the patients, unwanted hypotensive periods (< 70 mmHg) were registered during treatment, manageable with dosage adaption.

This evaluation shows that nicardipine is a potent antihypertensive drug and can be used for temporising management in severe, early-onset pre-eclampsia when other antihypertensive drugs have failed.

INTRODUCTION

The mainstay of treatment in pre-eclamptic patients is to lower the elevated blood pressure to prevent complications in the mother. While the ultimate cure for pre-eclampsia is delivery, studies have shown that temporizing management, using potent antihypertensive drugs, can improve neonatal outcome in severe early-onset pre-eclampsia [1-4].

Current options for antihypertensive treatment are limited. Worldwide, (di)hydralazine with its direct vasodilatating effect is used most often. In Europe, however, dihydralazin has recently been withdrawn from the market for economical reasons. The 5-HT_{2A} receptor antagonist ketanserin has been used increasingly, but according to both our experience and literature [5], the antihypertensive response is insufficient in a substantial number of patients and additional antihypertensive treatment is required.

The combined alpha and beta-blocking agent labetalol is available both orally and intravenously, but intravenous administration of high maternal dosages has been associated with severe neonatal cardiovascular adverse effects [6].

For oral use, methyldopa and the calcium-channel blocking agent nifedipine are available but these drugs lack potency as monotherapy in severe pre-eclamptic patients. Moreover, the lack of an intravenous drug dosage form precludes their application in acute situations.

Nicardipine, a dihydropyridine type calcium-channel antagonist, available both orally and parenterally, is a drug of interest for use in pre-eclamptic patients. The drug acts by blocking the transmembrane flow of calcium ions through voltage-gated L-type channels. This leads to a lower concentration of calcium in the cell and thereby diminishes contraction of vascular smooth muscle cells. The drug has been licensed for use in acute severe hypertension and postoperative hypertension during the first 24 hours following surgery, and it has shown a fast and potent lowering of the blood pressure after intravenous administration [7]. In comparison with nifedipine, nicardipine acts more selectively on blood vessels than on myocardial tissue, resulting in less reflex tachycardia. The short half life of the drug ($t_{½}$ = 2 - 5 min, increasing to 1 - 2 h after prolonged use [8]) enables an accurate titration of the blood pressure.

Studies with nicardipine in pre-eclamptic patients are scarce [9-12]. Only Carbonne et al [9] describes the succesful use of nicardipine for temporizing management in pre-eclamptic patients with a gestational age mainly above 32 weeks.

Our obstetric ward is a referral centre for early-onset pre-eclamptic patients (gestational age < 32 weeks) and the current policy is to use antihypertensive treatment in these patients to prolong pregnancy and improve neonatal outcome. However, failure of adequate antihypertensive blood pressure control occurs frequently under antihypertensive treatment and may necessitate delivery in very early pregnancy, with a corresponding high neonatal morbidity and mortality. Therefore, the need for new potent antihypertensive drugs in early-onset pre-eclamptic patients is apparent.

This observational study describes the use of nicardipine for temporizing management, when other therapeutic options have failed. Efficacy in terms of blood pressure control and postponement of delivery is assessed in a population of extensively pre-treated, early-onset pre-eclamptic patients. Safety issues for the mother, foetus and neonate are discussed.

METHODS

Patients, admitted to our High Care antenatal ward with severe pre-eclampsia in the period 2002 - 2004, were treated with intravenous nicardipine after failure of treatment with intravenous ketanserin or dihydralazin (in combination with methyldopa orally). Severe pre-eclampsia was defined as the occurrence after 20 weeks of gestation, of a diastolic blood pressure ≥ 110 mm Hg and proteinuria ≥ 0.3 g/l in a 24-h urine collection, or the occurrence of repetitive diastolic blood pressure > 90 mmHg in combination with Haemolysis, Elevated Liver Enzymes, Low Platelet Count (HELLP) syndrome. HELLP-syndrome was defined as the simultaneous occurrence of alanine aminotransferase and/or aspartate aminotransferase > 31 U/l (2 SD above the mean in our hospital), platelet count < 100 x 10^9 platelets/L and haptoglobin < 0.28 g/L. The diastolic blood pressure was measured at Korotkoff phase V with a mercury sphygmomanometer.

During admission, each woman received an indwelling arterial catheter for blood pressure measurement and a triple lumen central venous line for central venous pressure measurement and parenteral administration of medication. The central venous pressure was continuously maintained at 5-6 mmHg, using pasteurized plasma-solution. From 2004 onwards, magnesium sulphate treatment was administered prophylactically to all pre-eclamptic patients with a diastolic blood pressure ≥ 110 mmHg.

Antihypertensive treatment was continued as long as the foetal and/or maternal condition did not warrant delivery, as judged by the attending obstetrician. Foetal viability was set at 26 weeks gestation and a estimated foetal weight ≥ 650 g. When foetal viability was reached, the foetal condition was actively assessed, using cardiotocography (CTG) and taken into consideration in the decision to terminate the pregnancy. All patients received a course of antenatal corticosteroids after 26 weeks of gestation for foetal lung maturation.

Since this study comprises an evaluation of our current protocol regarding antihypertensive treatment using only observations and no interventions, no permission from the Institutional Review Board was required according to Dutch Law.

Drug treatment

Treatment was targeted at a diastolic intra-arterial blood pressure (DAP) of < 100 mmHg (or < 90 mmHg in patients with HELLP-syndrome) within 1 h after the start of treatment. All patients continued to use methyldopa orally in a maximum dosage of 4 g daily. The starting

dosage of nicardipine was 3 mg/h by continuous infusion through a central venous line. As soon as the target DAP was reached, the dosage was reduced. Nicardipine dosages were subsequently titrated according to the blood pressure with increments of 0.5 – 1 mg/h to a maximum of 10 mg/h.

Treatment was continued until maternal and/or foetal condition warranted delivery.

Data analysis

The efficacy of nicardipine was evaluated by assessing the number of patients that reached the target DAP and the time-interval needed to reach the target DAP. Treatment was considered a failure when target blood pressure could not be reached with nicardipine within 60 min. The prolongation of pregnancy with nicardipine treatment was determined until delivery or occurrence of intra-uterine foetal death. Safety of the therapy was assessed as the occurrence of hypotensive periods, defined as DAP < 70 mmHg and the occurrence of adverse maternal effects. Tachycardia was defined as the occurrence of a heart beat > 120 beats/min, and the dosage of nicardipine was adjusted when tachycardia was sustained for more than 20 min. Postpartum, the incidence of haemorrhage was registered (defined as blood loss after delivery of more than 500 ml).

Maternal side effects were registered daily by nurses, using a standardized questionnaire. The foetal condition was assessed by CTG in patients with a viable foetus. Possible influences of nicardipine dosage change or hypotension during nicardipine treatment on CTG parameters (appearance of decelerations and effect on baseline foetal heart rate) were retrospectively analysed visually by a single investigator without knowledge of the nicardipine dosage or DAP at the time of CTG interpretation.

Foetal and neonatal outcome was assessed in terms of intra-uterine foetal death, neonatal death (death within 6 weeks after birth), the number of severely growth-restricted neonates at birth, the pH value in the umbilical artery, the number of neonates with an APGAR score < 7 at 5 min, the number of neonates admitted to the intensive care unit, the number of neonates requiring artificial ventilation and the number of days of artificial ventilation, and the number of neonates with hypotension within 24 h after birth, needing to be treated with volume expansion and /or cardiovascular drugs.

RESULTS

Twenty-seven pre-eclamptic patients received nicardipine as second-line treatment, after failing first-line treatment with parenteral ketanserin (n=22), dihydralazine (n=2) or labetalol (n=1). Two patients received both ketanserin and dihydralazin, successively, before the start of nicardipine. The clinical characteristics of the study population are presented in Table 8.1. All patients but one continued or started on methyldopa orally, during nicardipine treatment.

The indication to start nicardipine was insufficient blood pressure control in 18 patients treated with ketanserin (maximum dosage, 20 mg/h) and in one patient treated with dihydralazin (maximum dosage, 9 mg/h). Three patients on ketanserin experienced side-effects necessitating a switch of therapy (hypotension and QT-interval prolongation respectively), whereas one patient on dihydralazin experienced hypotension. One patient was started on ketanserin in combination with methyldopa, but, due to the occurrence of hypotension, ketanserin was stopped. Monotherapy with methyldopa eventually was not sufficient to keep adequate blood pressure control, and nicardipine was added. In two patients nicardipine was started after both ketanserin (insufficient effect and bradycardia, respectively) and, subsequently, dihydralazin (general malaise and unstable blood pressure, respectively) were stopped. One patient was switched from labetalol to nicardipine due to lack of efficacy.

In 22 patients CTG were obtained during nicardipine treatment. Two other patients were below 26 weeks gestation, and the remaining three patients had incomplete data.

Table 8.1. Clinical characteristics of pre-eclamptic patients (n=27) treated with nicardipine as second-line treatment

	Number (%) or Median (range)
Systolic blood pressure (mm Hg)	
- At admission[1]	175 (130-220)
- At start nicardipine[2]	191 (151-224)
Diastolic blood pressure (mm Hg)	
- At admission[1]	110 (80-130)
- At start nicardipine[2]	104 (80[3]-123)
Proteinuria at admission (g/l)	1.94 (0.15-34.7)
Maternal age (years)	31 (18-41)
Gestational age (weeks)	
- At admission	26[4] (21[1] - 31 [2])
- At start nicardipine	27[1] (21[2] – 32[4])
Nulliparous (n)	19 (70%)
Pre-existent hypertension (n)	6 (22%)
HELLP syndrome (n)	
- At admission	8 (30%)
- At start nicardipine	9 (33%)
Oral antihypertensive co-medication at admission (n)	
- Methyldopa	25 (92%)
- Nifedipine	2 (7%)
- Labetalol	3 (11%)
- None	1 (4%)
First-line intravenous antihypertensive treatment (n)	
- Ketanserin	22 (81%)
- Dihydralazin	2 (7%)
- Ketanserin and dihydralazin	2 (7%)
- Labetalol	1 (4%)

[1] manually measured, [2] intra-arterially measured, [3] Two patients with adverse effects on first-line treatment

Efficacy

In all patients with elevated blood pressure at time of start of nicardipine, the target DAP was obtained within a median period of 23 min (range 5 - 60 min) using nicardipine. No treatment failures occurred. In three patients the starting dosage of nicardipine was below 3 mg/h because of border-line hypertension before start of nicardipine [2 mg/h (n=1), 1 mg/h (n=2)]. All other patients started with intravenous nicardipine in a dosage of 3 mg/h. In three patients a temporary increase of the dosage to 4 mg/h was necessary to obtain target blood pressure initially. After reaching the target blood pressure, the dosage of nicardipine was reduced by 0.5, 1 or 2 mg/h in all patients.

In nine patients we performed continuously arterial sampling of systolic, diastolic and mean arterial blood pressure and the heart rate during the first 90 min of nicardipine infusion. These measurements are shown in Figure 8.1a-d.

Figure. 8.1 Haemodynamic characteristics of pre-eclamptic patients before and after start of nicardipine infusion (n=9)

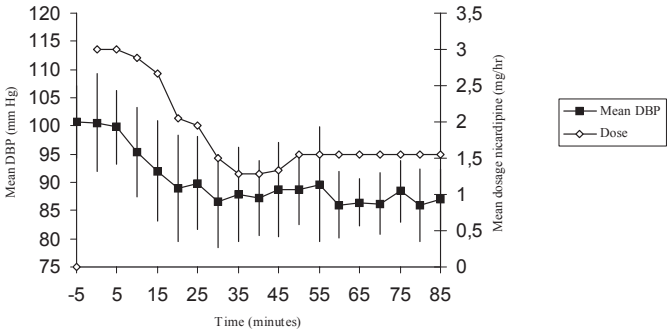

Fig. 8.1a Mean (standard deviation) diastolic arterial blood pressure (DBP) and corresponding nicardipine dosage

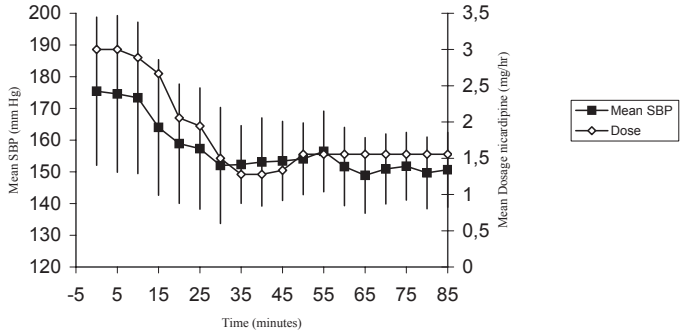

Figure. 8.1b Mean (standard deviation) systolic arterial blood pressure (SBP) and corresponding nicardipine dosage

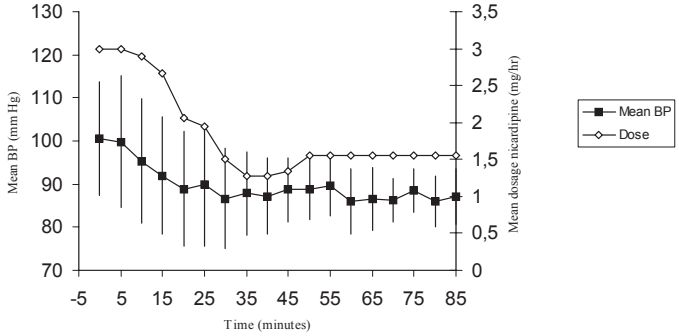

Figure 8.1c Mean (standard deviation) arterial blood pressure (BP) and corresponding nicardipine dosage

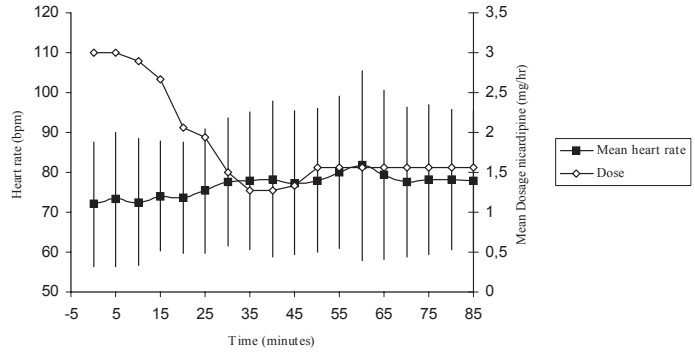

Figure. 8.1d Mean (standard deviation) heart rate and corresponding nicardipine dosage

Twenty-six patients received nicardipine treatment until delivery. In one patient nicardipine was stopped after 3.5 days because of a lack of need for continuing intravenous antihypertensive treatment. In this patient, treatment was switched to nifedipine orally.

The maximum dosage of nicardipine during treatment ranged from 3 to 9 mg/h, with a median of 4 mg/h.

Maternal outcome is summarized in Table 8.2. Foetal death occurred in three patients; in two women at 24 weeks of gestation and in one women at 27[5] weeks of gestation. In all three patients a non-intervention policy was followed because of the early gestational age or a severely growth-restricted foetus with a poor prognosis; in these patients only the maternal condition and not the foetal condition, was taken into consideration in the decision to deliver the mother.

In our cohort, pregnancy was prolonged for a median of 4.7 days (median 1 - 26 days) under nicardipine treatment, after failure of earlier intravenous antihypertensive treatment. Most women (74%) were delivered because of deteriorating foetal condition. In four patients the maternal condition was the reason for delivery.

Using the dosage schedule as already described, no patients experienced inadequate blood pressure control during nicardipine treatment. However, in the first months after implementing the protocol, oral metoprolol was added during nicardipine treatment in five patients when nicardipine dosages were 7 mg/h or higher. Due to the limited experience with nicardipine at that time, the attending physician felt the need to add metoprolol, to ensure adequate blood pressure control in the long term. During the course of the study, this practice was not deemed necessary anymore.

Table 8.2. Maternal outcome after treatment with nicardipine for pre-eclampsia (n=27)

	Number (%) or Median (range)
Gestation age at delivery or at occurrence of IUFD (weeks)	27[5] (24[4] - 34)
Target diastolic blood pressure within 60 min	27 (100%)
Prolongation of pregnancy on nicardipine (days)	4.7 (1-26)
Caesarean section (n)	24 (89%)
Indication for delivery (n)	
- Foetal distress	20 (74%)
- Maternal condition	4 (15%)[a]
- IUFD	3 (11%)
Complete antepartum resolution of HELLP-syndrome during nicardipine treatment (n)	4 (15%)

IUFD, intra-uterine foetal death; [a] Respectively fever, dyspnoea, severe subcutaneous oedema and pulmonary oedema after fluid overload.

Safety

In eight patients (30%) short episodes of hypotension (DAP < 70 mmHg) were registered. In two patients the hypotension necessitated a reduction of the dosage of nicardipine, and in three patients nicardipine was temporarily withdrawn, leading in all patients to a DAP > 70 mmHg. In the remaining patients, DAP < 70 mmHg was accepted based on the clinical condition of the mother. No patients suffered a hypotensive episode severe enough to require delivery. Tachycardia (heart rate > 120 beats/min) occurred in five (18%) patients during nicardipine treatment, but since the observed tachycardia was sustained for a period shorter than 20 min, no stopping or lowering of the dosage of nicardipine was necessary.

From 2004 onwards, magnesium was used as prophylaxis against seizures in pre-eclamptic patients. Three patients in our study received magnesium during nicardipine treatment, without showing any effect on the blood pressure.

The side effects most frequently reported by the patients were fatigue, dry mouth, stuffy nose, lost of appetite and constipation. One patient experienced severe diarrhoea and in one patient serum urea was increased (during steroid treatment). Because these effects could not be excluded as caused by nicardipine, intravenous ketanserin was added and the dosage of nicardipine was not increased.

In one patient with intra-uterine foetal death at 24 weeks, induction of labor with prostaglandine E_2 (Sulproston) took several days to be successful, which may be (partly) attributed to a tocolytical effect of nicardipine. Haemorrhage postpartum (defined as > 500 ml blood loss) occurred in six patients (27%) and blood transfusions were needed in five of these patients. This is high in comparison with historical data from 254 pre-eclamptic patients treated at our unit with dihydralazin, in which haemorrhage postpartum occurred in 15 patients (6%) [1].

Foetal safety

Two patients showed the appearance of variable decelerations within 1 h after hypotension during nicardipine treatment. Intervention for foetal distress was not indicated. Decelerations disappeared spontaneously in both patients. Both patients also had variable decelerations before starting nicardipine.

Raising the nicardipine dosage resulted in the appearance of decelerations (within 2 h) in four patients. None of these patients were hypotensive. Intervention for foetal distress was indicated once in a patient with a systolic blood pressure of 190 mmHg and a diastolic blood pressure of 110 mmHg. The other patients had no change in therapy, and decelerations resolved spontaneously. All patients also had variable decelerations before starting nicardipine.

Dosage reduction resulted in appearance of decelerations in four patients (total of five episodes). None of these patients were hypotensive. One patient again had dosage reduction, but change of treatment in other patients was not indicated and decelerations resolved spontaneously. All patients also had variable decelerations before starting nicardipine. No changes in the baseline foetal heart rate were observed.

Neonates

Neonatal outcome is presented in Table 8.3. Two neonates died within 6 weeks after birth. One neonate died after 6 days of severe idiopathic respiratory distress syndrome and the other neonate died 17 days after birth after relaparotomy because of necrotizing enterocolitis.

Table 8.3. Foetal (n=27) and neonatal (n=24) outcome after maternal treatment with nicardipine for pre-eclampsia

	Number (%) or median (range)
Number of foetuses (n)	27
Foetal death	3 (11%)
Number of neonates (n)	24 (89%)
Gestational age at birth (weeks)	28^{2} (25^{6} - 34)
Birth weight (g)	925 (560 - 1685)
Growth percentile (n)	
- Below 10% percentile	11 (46%)
- Below 2.3% percentile	2 (8%)
pH value umbilical artery	7.23 (7.06-7.38)
Apgar score <7 at 5 min (n)	2 (8%)
Neonatal death (< 6 weeks)	2 (8%)
Neonatal care (n)	
- Intensive Care	21(88%)
- days on Intensive Care	15.5 (1-70)
- Medium Care	3 (12%)
- No admission	0
Mechanical ventilation (n)	18 (75%)
- Days on mechanical ventilation	8 (1-39)
Hypotension within 24 h after birth, needed to be treated with volume expansion and /or cardiovascular drugs (n)	8 (33%)

DISCUSSION

In severe pre-eclamptic patients, aggressive antihypertensive treatment should be used to stabilize the condition of the mother. Furthermore, delivery can sometimes be postponed, while corticosteroids are administered to the mother to improve foetal lung maturation. There is still a lot of debate whether prolonged antihypertensive treatment in early-onset pre-eclampsia results in improved neonatal outcome [13-16], but in our hospital the current policy is to use temporizing antepartum management with parenteral antihypertensive treatment in early-onset pre-eclampsia, as long as the foetal and maternal condition allows.

Parenteral antihypertensive treatment should be administered under close monitoring of the mother and foetus. Preferably, continuous arterial blood pressure measurements and central venous pressure monitoring should be carried out in each patient in a high-care obstetric setting. Inadequate maternal blood pressure control or an overshoot of blood-pressure-lowering therapy, resulting in foetal distress, can compromise a safe delivery.

However, therapeutic options with respect to antihypertensive treatment are limited and far from optimal with respect to efficacy and side-effects [5]. Therefore, alternative drugs that are effective and safe for both mother and child are sorely needed.

In studies in pregnant rhesus monkeys and sheep [17-19], the calcium-channel blocking agents nifedipine and nicardipine have been associated with foetal acidemic responses and

concern has risen for treatment with dihydropyridine derivates in pregnant women. However, extensive experiences with nifedipine as a tocolytical drug in pregnant women have not revealed any negative effects on foetal or uteroplacental circulation [20].

Nicardipine has less effect on myocardial function than nifedipine, and its fast and easily controllable antihypertensive effect makes the drug useful for acute hypertensive emergencies. Haemodynamic effects of short-term infusions of nicardipine in pre-eclamptic patients have been described in the literature [10-11], resulting in a fast and effective decrease of blood pressure in all patients.

Carbonne et al. [9] reported the successful use of intravenous nicardipine in a fixed dosage-schedule for prolongation of the pregnancy for a mean of 5.3 ± 3.6 days in 20 pre-eclamptic patients (gestational age 33 ± 3.6 weeks), and no adverse effects on foetus or neonates were found. Seki et al. [11] demonstrated that prophylactic treatment with nicardipine in patients with severe hypertension during pregnancy was both effective and safe for mother and child.

However, since data regarding long-term efficacy in pre-treated, early onset pre-eclamptic patients are lacking, the current observational study was carried out to determine efficacy of nicardipine, after failure of first-line antihypertensive treatment.

Efficacy

We demonstrate in our study that nicardipine is an effective drug in obtaining and maintaining blood pressure control in severe, early-onset pre-eclamptic patients, even when other antihypertensive treatment has failed. Within a median period of 20 min after the start of the drug, patients reached target blood pressure and no treatment failures occurred. In comparison with the 5-HT_{2A} antagonist ketanserin, which was shown to be ineffective in a substantial part of pre-eclamptic patients [5], these results indicate a high efficacy profile for nicardipine, even in our population of non-responsive pre-treated patients. Adequate blood pressure control using nicardipine was maintained for a period of days in most patients and pregnancy was prolonged for a clinically significant period of time under nicardipine treatment. Maternal condition was the reason for delivering the patient only in a minority of patients, indicating that the drug is effective and tolerated well by the mother.

As shown by the continuous blood pressure measurements after the start of the infusion, the antihypertensive effect of the drug was easily manageable by titration of the dosage, due to the fast onset of antihypertensive effect and short half-life of the drug.

However, some characteristics of nicardipine, such as its high intrinsic potency, the extensive metabolism by the liver and the side-effects like tachycardia and tocolytic effects, might be disadvantageous in pre-eclamptic patients. These safety issues have been looked upon specifically in our patients.

Hypotension

In pre-eclamptic patients, the risk of compromising the foetal condition by aggressive lowering of maternal blood pressure has to be weighted against the need to protect the mother from complications due to a highly elevated blood pressure.

The widely used antihypertensive drug (di)hydralazin is known to cause foetal distress because of the unpredictable fall in maternal blood pressure, especially when circulating volume is low and in circumstances where adequate monitoring of maternal blood pressure and pulmonary capillary wedge pressure or central venous pressure is not feasible [6].

In our study, an unwanted hypotensive period (defined as DAP < 70 mmHg) occurred during the use of nicardipine in one fifth of the patients. Although these hypotensive periods did not result in acute foetal distress or emergency delivery, intervention in treatment (reduction of dosage or temporarily stopping of the treatment) was deemed necessary in these patients. This shows that the current dosage schedule of nicardipine might not be optimal in all patients and that more studies are needed to evaluate the relationship between the dosage of nicardipine and antihypertensive effects in individual pre-eclamptic patients.

HELLP-syndrome

Nicardipine is metabolised extensively by the liver and therefore, in non-pregnant patients with liver function disorders, administration with caution is advised [8]. The occurrence of HELLP-syndrome in pre-eclamptic patients might influence the pharmacokinetic parameters of nicardipine, and more data in this subpopulation are needed. Our observation that the HELLP-syndrome was resolved under nicardipine treatment in 15% of the patients is in agreement with the fact that resolution of HELLP-syndrome during temporizing management of pre-eclampsia is known to occur [16].

Heart rate

The increase in heart rate under nicardipine treatment is a common side effect in all calcium-channel blocking agents of the dihydropyridine type, as well as with (di)hydralazin, and is caused by a reflex response to systemic arterial vasodilatation. The tachycardia was not considered a clinical problem in our patients by the treating physician, but almost all patients in our evaluation used methyldopa as co-medication as part of the protocol, which may have been beneficial because of its slowing effect on heart rate.

Combination with magnesium sulphate

Simultaneous use of magnesium sulphate for prophylaxis of seizures with nicardipine has been associated with an excessive hypotensive response, due to a combined blocking effect on calcium entry in the cell [21]. In our evaluation, magnesium sulphate was administered during nicardipine treatment in three patients, but in none of these patients an increase in antihypertensive response was seen.

Tocolytical effects

Nicardipine, as well as nifedipine, has been shown to possess tocolytical effects, because of its relaxing effect on smooth muscle contractility [22]. Obviously, induction of labor can be more difficult if a tocolytical (side) effect of nicardipine is present, as was seen in one of our patients after intra-uterine foetal death. After delivery, the risk on haemorrhage may be increased, because of uterine atonia. In our evaluation, no major clinical problems were experienced, but the increased incidence of haemorrhage postpartum, as compared with our historical data [1], suggest that possible inhibitory effects of nicardipine on postpartum uterine contractions may indeed result in an increased risk of haemorrhage postpartum.

Foetal outcome

Nicardipine dosage change or hypotension during nicardipine treatment did not result in an increase in appearance of deceleration, which needed treatment. No foetal distress caused by nicardipine occurred. All patients with decelerations already had transient decelerations before onset of nicardipine, suggesting other causes of decelerations such intra-uterine growth restriction or placental dysfunction.

Neonatal outcome

Our neonatal data are comparable with respect to APGAR scores and umbilical core pH to our own data [23] from neonates after temporising management with only ketanserin and/or dihydralazin, indicating the lack of any effect of nicardipine on neonatal acid-base status at delivery.

Transplacental transmission of nicardipine after maternal use is thought to be limited [9]. However, pharmacological effects of nicardipine in the neonate after maternal use cannot be excluded, and our incidence of hypotension in one-third of the neonates might in part be attributed to maternal use of nicardipine.

From our observational study, the data on neonatal morbidity and mortality are difficult to interpretate without a control group of neonates of pre-eclamptic mothers with comparable gestational age and temporizing management, preferably treated in the same hospital in the same time-period. However, within the limitations of this setting, perinatal and neonatal outcome did not seem compromised for our group of patients, especially when taken into account the pre-selected patient-population with failure of first-line antihypertensive treatment.

For comparative efficacy and safety with other antihypertensive drugs in pre-eclamptic patients, randomised clinical trials are warranted.

In conclusion, this observational study confirms that nicardipine is a potent antihypertensive drug, and in addition shows that nicardipine can be used effectively for temporizing management in early-onset pre-eclampsia when other antihypertensive drugs have failed.

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Chapter 9

Nicardipine in pre-eclamptic patients: Transplacental transfer and disposition in breast milk

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ABSTRACT

To assess the safety risks on the foetus and neonate caused by maternal use of nicardipine in pre-eclamptic patients, we evaluated the transplacental transfer and the transfer to breast milk after maternal intravenous administration of nicardipine. In ten pre-eclamptic patients nicardipine concentrations of maternal blood (P) and both arterial and venous umbilical cord blood samples ($U_{arterial}$ and U_{venous}) were assessed and the ratio of maternal and foetal plasma concentrations was calculated as an indication of transplacental transfer. We found a median transfer of 0.15 ($U_{arterial}$/P, range 0.05-0.22), and 0.17 (U_{venous}/P, range 0.023 – 0.22). The concentration nicardipine in umbilical cord was correlated with the maternal dosage of nicardipine at delivery (arterial: r_s=0.857 (p=0.007), venous: r_s=0.800 (p=0.005)). The highest umbilical cord concentration, found after a maternal dosage of 4.5 mg/h was 18 ng/ml, which can be considered as subtherapeutic. Therefore foetal adverse reactions caused by a direct pharmacological effect of nicardipine are unlikely to occur.

Nicardipine levels were determined in 34 breast milk samples of seven patients and were found to be undetectable in 82% of the samples. In six breast milk samples of four different patients, nicardipine levels (ranging from 5.1 to 18.5 ng/ml) were detectable during maternal nicardipine dosages ranging from 1 to 6.5 mg/h. The maximum possible exposition of a neonate to nicardipine was calculated to be less than 300 ng/day, which is an insignificant fraction of the therapeutic dosages used in neonates.

In conclusion, the exposition of the foetus and neonate to nicardipine through transplacental transfer and disposition in breast milk expression is low.

INTRODUCTION

The main goal of treatment of severe pre-eclampsia is to lower the elevated blood pressure to prevent complications to the mother and, in early onset pre-eclampsia, to prolong the pregnancy to improve the outcome of the foetus. The ideal antihypertensive drug for the treatment of severe hypertension in pre-eclampsia should be potent, rapidly acting controllable and with a limited placental transfer. Unfortunately, as yet no such drugs are available.

Intravenous administration of the calcium-channel blocking agent nicardipine has effectively been used as temporising management in pre-eclamptic patients [1]. The drug has shown a high potency in lowering maternal blood pressure, while its fast onset of action and its short elimination half-life (2-5 min) enables accurate blood pressure control. The use of calcium-channel blocking agents with a dihydropyridine structure, such as nifedipine and nicardipine, has for a long time been considered hazardous in pregnant women because of studies showing foetal acidemic responses in pregnant rhesus monkey and sheep [2,3]. However, extensive experiences with nifedipine as a tocolytical drug in pregnant women have not revealed any negative effects on the uteroplacental circulation [3]. Foetal and neonatal outcome data after maternal use of nicardipine are more scarce, but outcomes seem comparable with those of neonates from pre-eclamptic patients, treated with other antihypertensive drugs [1].

Still, maternal use of nicardipine may expose the foetus to pharmacologically active concentrations of the drug by transplacental transfer, whereas postnatally the neonate may be exposed to nicardipine during lactation. Because data regarding transplacental transfer and transfer into breast milk are lacking, we determined the transplacental transfer of nicardipine and disposition of nicardipine in breast milk.

METHODS

Patients with severe pre-eclampsia were treated at the Obstetric High Care unit at our antenatal ward with intravenous nicardipine in 2004 - 2005 as second-line treatment, after failure of treatment with intravenous ketanserin or dihydralazin (in combination with methyldopa orally). Severe pre-eclampsia was defined as the occurrence after 20 weeks of gestation, of a diastolic blood pressure ≥ 110 mmHg and proteinuria ≥ 0.3 g/l in a 24 hours urine collection, or the occurrence of repetitive diastolic blood pressure > 90 mmHg in combination with the HELLP-syndrome. HELLP-syndrome was defined as ALAT and/or ASAT > 31 U/l, platelet count below 100 x 10^9 platelets/L and haptoglobin below 0.28 g/l. At admission, diastolic blood pressure was measured at Korotkoff phase V with a mercury sphygmomanometer. During admission patients received a radial arterial line for

intra-arterial blood pressure. Target diastolic arterial blood pressure (DAP) with nicardipine treatment was < 100 mmHg (< 90 mmHg for patients with HELLP-syndrome) measured intra-arterially. Treatment with nicardipine started with continuous intravenous infusion of 3 mg/h and as soon as target DAP was reached, the dosage was reduced. Nicardipine dosages were subsequently titrated according to the blood pressure with increments of 0.5 - 1 mg/h to a maximum of 10 mg/h. Nicardipine treatment was continued as long as the foetal and/or maternal condition did not warrant delivery, as judged by the attending obstetrician. The study protocol was reviewed and approved by the Institutional Review Board of the Erasmus MC and all patients gave informed consent.

Placental transfer and transfer into breast milk

To determine the placental transfer, a maternal blood sample was drawn just before delivery, and umbilical cord blood samples, both arterial and venous, were drawn just after delivery. Both arterial and venous concentration levels were studied to obtain information with respect to foetal metabolism of nicardipine.

To determine exposure during lactation, breast milk was collected from patients until 24 hours after stopping of the administration of nicardipine after delivery. After each breast milk collection, a maternal blood sample was drawn.

Nicardipine levels in plasma and breast milk samples were determined using a validated reversed-phase high performance liquid chromatographic assay with UV detection, developed at our hospital pharmacy. The lower limit of quantification (LOQ) of nicardipine in plasma and in breast milk was 1.4 ng/ml and 5.0 ng/ml respectively. The interday variation (reproducibility) for the concentrations 10 ng/ml, 50 ng/ml and 100 ng/ml for plasma were 4.6%, 2.1%, 3.9% respectively, and for breast milk 6.5%, 4.8% and 4.2% respectively. Plasma samples and breast milk samples were stored at -18 ºC until analysis.

Data analysis

The concentration ratios of nicardipine in umbilical cord plasma (both arterial and venous ($U_{a,v}$)) versus maternal plasma (P) were used as a measure of transplacental transfer. The concentration ratios of nicardipine in breast milk (M) versus the maternal plasma (P) were used as a measure of transfer to breast milk (M/P). Multiplying the volume of every breast milk portion with the concentration of the corresponding sample and subsequently totalling the absolute amounts of nicardipine for each day, calculated the daily exposure of a neonate to nicardipine during lactation.

The differences between arterial umbilical cord concentrations and venous umbilical cord concentrations were tested, using the Wilcoxon signed rank test (p-level ≤ 0.05). Correlations between transplacental transfer and maternal dosage at delivery and total cumulative dosage respectively, were tested using the Spearman's rank correlation test (p-level ≤ 0.05).

RESULTS

Placental transfer

Ten patients were included. The patient characteristics are summarised in Table 9.1. Nicardipine dosage at time of delivery ranged from 1 to 7 mg/h. Cumulative dosage until delivery ranged from 4.5 – 450 mg. Median duration of treatment with nicardipine until delivery was 55 hours (range, 1.5-100 hours) and the maximal dosage during treatment varied from 1 to 9 mg/h. Eight patients had the same dosage nicardipine during 6 hours before delivery. One patient started with nicardipine 3 mg/h 4 hours before delivery and after two hours the dosage was lowered to 1.5 mg/h until delivery. Considering the fact that a steady state will have been reached by time of delivery, given the short elimination half-life of nicardipine, this patient was included in the analysis. One patient started with nicardipine 3 mg/h 1.5 hours before delivery and stopped nicardipine just before delivery. Maternal plasma concentrations dropped quickly, while the nicardipine concentration in the fetal compartment was still high, resulting in a high U/P ratio of 0.5. The plasma concentrations of this patient did not reflect a steady-state situation and this patient was excluded in further analysis. For one patient arterial and venous umbilical cord blood was accidentally pooled and for one patient only venous umbilical cord blood was drawn.

Table 9.1 Maternal and neonatal characteristics. Values are expressed as n (%) or mean (range)

Maternal characteristics (n=10)	
Maternal age (years)	28 (17-36)
Gestational age at admission (weeks)	28 3/7 (25 5/7 – 32 4/7)
Gestational age at start nicardipine (weeks)	28 4/7 (26 3/7 – 31 4/7)
Nulliparity (n)	5 (50%)
Systolic bloodpressure at admission (mmHg)	190 (160 – 220)*
Diastolic bloodpressure at admission (mmHg)	115 (100 – 130)*
HELLP at admission (n)	2 (20%)
Caesarian deliveries	9 (90%)
Vaginal deliveries	1 (10%)
Indication for delivery	
- Foetal distress (n)	6 (60%)
- Maternal distress (n)	4 (40%)
Neonatal outcome (n=10)	
Perinatal deaths	0 (0%)
pH umbilical cord	7,29 (7,08 – 7,42)
Apgar < 7 at 5 min (n)	0 (0%)
Birthweight (g)	980 (630 – 1660)
Admission	
- intensive care unit	9 (90)
- medium care	1 (10)
ICU stay (days)	16 (4-56)

*Measured with cuff

Nicardipine could be detected in all maternal and umbilical cord blood samples. Maternal nicardipine concentration levels ranged from 9.8 to 116 ng/ml. In one umbilical cord sample nicardipine concentration was below the limit of quantification (LOQ), for further calculation we assumed the concentration being 1.4 ng/nl (LOQ). Venous umbilical cord nicardipine concentrations ranged from 1.4 to 18.6 ng/ml and arterial umbilical cord nicardipine concentrations ranged from 1.4 to 15.7 ng/ml.

The calculated median transplacental transfer was 0.15 ($U_{arterial}$/P, range 0.05-0.22), and 0.17 (U_{venous}/P, range 0.023 – 0.22). No differences could be detected between arterial and venous umbilical cord concentrations (p = 0.15).

A significant correlation could be demonstrated between maternal dosage at delivery and arterial and venous umbilical blood concentrations (r_s=0.857 (p=0.007) and r_s=0.800 (p=0.005, Figure 9.1). No correlation was found between total cumulative dosage until delivery and $U_{arterial}$ /P (r_s=0.429 (p=0.169) and U_{venous}/P (r_s=0.350 (p=0.178).

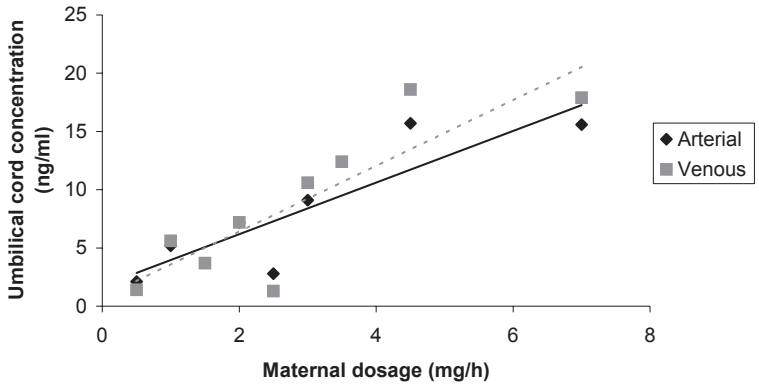

Figure 9.1. Maternal nicardipine dosage at delivery versus umbilical cord nicardipine concentration, both arterial and venous. The correlation between maternal nicardipine dosage and umbilical cord concentration and arterial and venous umbilical blood concentration was found to be r_s=0.857 (p=0.007) and r_s=0.800 (p=0.005) respectively.

Transfer in breast milk

34 breast milk samples were obtained of seven patients, using nicardipine after delivery. Median gestational age at delivery was 28[6] weeks (26[1] – 33). After delivery intravenous nicardipine was continued for a median of 1.9 days (range 0.8 - 4.6 days). The maximum dosage of intravenous nicardipine during lactation was 6.5 mg/h.

In 82% of the breast milk samples, no nicardipine could be detected and therefore the median M/P ratio could not be determined. In 6 breast milk samples of four different patients nicardipine levels (ranging from 5.1 to 18.5 ng/ml) were detectable during maternal

nicardipine dosages, ranging from 1 to 6.5 mg/h. Because of the limited data, no analysis could be performed to investigate the influence of maternal drug dosage and breast milk concentrations.

The highest concentration of nicardipine determined in breast milk was 18.5 ng/ml, during maternal nicardipine use of 5.5 mg/h intravenously. The maximum possible exposition of a neonate to nicardipine during lactation was calculated to be below 300 ng/day. In comparison, intravenous dosages of nicardipine used for treating preterm neonates with hypertension are 0.72 - 2.9 mg/kg/day [4], which correspond to an oral dosage of 2 mg - 8.3 mg/kg/day, assuming an oral bio-availability of 35% [5]. The possible exposure of 300 ng/day in breast milk corresponds therefore to 0.015% to 0.004% of a therapeutically dosage of nicardipine in a one-kg neonate.

DISCUSSION

While using drugs in pregnant women, data with respect to placental transfer are essential to assess the risk on adverse effects on foetus and neonate. In this study transplacental transfer and disposition in breast milk of nicardipine was assessed after maternal nicardipine use for antihypertensive treatment of pre-eclampsia.

Our study shows that transplacental transfer of nicardipine is low, resulting in subtherapeutic concentrations in umbilical cord blood. Carbonne et al [6] have described the use of nicardipine in pre-eclamptic patients. From their data on maternal and foetal plasma concentrations in two patients, the transplacental transfer can be calculated (0.17 and 0.11, respectively), which is comparable with our results.

Studying placental transfer of drugs by comparing umbilical cord blood concentration with maternal blood concentration is a well established approach [7]. A limitation of this method is that only a single set of data is available for each patient, but the results do yield important information regarding in vivo transfer and elimination of a drug to and from the foetus.

Passive diffusion is the main mechanism of transplacental transfer of drugs. We found that higher maternal dosages of nicardipine at delivery result in higher concentrations of nicardipine in the umbilical artery. This may agree with a placental transfer of nicardipine by diffusion, which is characterised by a transfer, proportional to the concentration gradient between maternal and foetal plasma. Key factors in this mechanism are the physiochemical properties of the drug such as protein binding, pKa, molecular weight and lipophilicity [8]. Because only the unbound fraction of nicardipine is available for placental transfer, the high protein-binding (98%) [9] of nicardipine is probably one of the main causes for the low transplacental transfer. Additionally, being a weak base with a pKa of 7.2, the main part of nicardipine is protonized at the physiological maternal pH, reducing its availability for placental transfer.

Another explanation for the low placental transfer of nicardipine might be that metabolism in the placenta occurs before the drug reaches the foetus. Nicardipine is thought to be a substrate to P-glycoprotein [10] and placental P-glycoprotein is known to provide a mechanism of inhibiting placental transfer of xenobiotics that are substrates for his efflux pump. This may have contributed to the low nicardipine levels in the umbilical cord.

The low transplacental transfer of nicardipine is in contrast with that of other antihypertensive drugs used in pre-eclampsia such as hydralazin, nifedipine, methyldopa and labetalol which are known to pass the placenta extensively [11,12]. This can be considered an important advantage for the use of nicardipine during pregnancy.

We found no correlation between total cumulative dosage until delivery and transplacental transfer, suggesting that nicardipine does not cumulate in the foetus. Accumulation in the foetus may be caused by ion- trapping of nicardipine following a higher proportion of ionized drug in the more acidic foetal circulation as compared to the maternal circulation, but our results do not support this.

No difference between arterial and venous umbilical cord concentrations was found. This may be related to the relatively immature foetal hepatic metabolising capacity.

The highest umbilical cord nicardipine level, detected in our patients, was 18 ng/ml. For adults nicardipine levels of 70-100 ng/ml are considered therapeutic [13]. Holbrook et al [14] investigated direct nicardipine infusion in foetuses of ewes, which resulted in mean foetal plasma concentrations of respectively 78 ng/ml and 114 ng/ml. No significant changes of foetal heart rate, foetal arterial blood gas values were observed at these concentration levels. Although animal data cannot be directly extrapolated, our nicardipine concentrations found in human umbilical cord blood indicate a low risk for a direct pharmacological effect on the human foetus.

Our data showing absence of nicardipine into the majority of breast feeding samples indicate a low transfer in breast milk. Prediction of transfer to breast milk based on physicochemical properties is known to be unreliable [15], still, a relatively low transfer was expected because of the high protein binding of nicardipine. Nifedipine, structurally similar to nicardipine also has a low transfer in breast milk [16]. The limited transfer into milk in combination with an oral bioavailability of 35% of nicardipine, indicate that exposure of the neonate to nicardipine during lactation will be low.

In conclusion, our results showing limited transplacental transfer and low disposition in breast milk support the use of nicardipine as antihypertensive drug in pre-eclampsia.

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Chapter 10

General discussion and suggestions for future research

INTRODUCTION

Pre-eclampsia is a disease, exclusively occurring in pregnant women and characterised by the occurrence after the 20th week of gestation of maternal elevated blood pressure and proteinuria. Serious maternal complications can occur, such as HELLP-syndrome (haemolysis, elevated liver enzymes, low platelet counts), pulmonary oedema, liver failure or haemorrhage, abruptio placentae, eclampsia and even cerebral haemorrhage and maternal death. The associated placental dysfunction may result in intrauterine growth restriction and iatrogenic preterm delivery of small for gestational age babies and perinatal death [1].

In a tertiary care referral centre for severe early-onset pre-eclamptic patients, like the Obstetric and Prenatal Medicine Department of the Erasmus MC, the goal is to stabilise the patient using antihypertensive treatment and postpone delivery as long as foetal and maternal condition allow, to improve neonatal outcome. To achieve this goal, long-term use of often high dosages of antihypertensive drugs is usually necessary, but a delicate balance between the optimum treatment for the mother on one hand and the well-being of the foetus on the other hand needs to be maintained.

Because of decreasing availability of adequately studied drugs in pre-eclampsia and recent controversy surrounding the risks and benefits of the current standard drug (di)hydralazin, the need for information on alternative drugs became apparent. To fill this gap, studies on several aspects, regarding efficacy and safety of antihypertensive treatment with ketanserin and nicardipine in pre-eclampsia, are reported in this thesis.

Ketanserin

Ketanserin is one of the two drugs licensed for treatment of hypertension in pregnancy in the Netherlands. In the late nineties, ketanserin became the first choice treatment for the management of pre-eclampsia in our hospital as well as in many other hospitals in the Netherlands. The drug acts by blocking the vasoconstrictive response upon binding of serotonin (5-HT) to 5-HT_{2A} receptors in vascular tissue. Pre-eclampsia is characterised by endothelial dysfunction and due to the concomitantly loss of 5-HT_{1} endothelial receptors, free serotonin will stimulate mainly 5-HT_{2A} receptors in platelets causing platelet aggregation and serotonin release, and 5-HT_{2A} receptors in vascular smooth muscle, causing vasoconstriction. Ketanserin acts as an antagonist on the 5-HT_{2A} receptor, counteracting serotonin dependent vasoconstriction and platelet aggregation. The latter may especially be important in pregnancies complicated with the HELLP-syndrome [2].

Pharmacokinetic aspects

Following our observations that in clinical practice many of our early-onset pre-eclamptic patients do not respond or only temporarily respond to ketanserin, we studied the possible causes for the limited efficacy of ketanserin.

Pharmacokinetic problems such as inadequate drug levels at the receptor-site may cause efficacy problems. During pregnancy, large physiological changes are known to occur (such as increased plasma volume, decreased serum albumin, increased glomerular filtration rate and altered hepatic metabolism) which can affect concentrations of drugs in pregnancy [3]. In pre-eclamptic patients, some of these features are known to differ from normal pregnancies (e.g. decreased plasma volume and decreased glomerular filtration) [4]. Large intra-individual differences occur in pre-eclamptic patients with respect to bodyweight, amount of oedema, blood pressure at presentation, hepatic and renal involvement and occurrence of HELLP-syndrome, which can be speculated to result in differences in clearance and volume of distribution of drugs. This variety in clinical presentation may necessitate a more individual approach in management, as it may be the cause of insufficient efficacy in pre-eclamptic patients following a fixed dosage schedule of an antihypertensive drug.

Our results of therapeutic or even supratherapeutic levels of ketanserin in all our pre-eclamptic patients and the absence of differences in pharmacokinetic parameters between our pre-eclamptic population and non-pregnant volunteers, indicate that pharmacokinetic differences, however, do not explain the lack of efficacy. The currently used dosage schedule apparently yield pharmacologically active blood levels, at which antagonism of the 5-HT_{2A} receptor should occur. Furthermore, our data also indicate that increasing the dosage of ketanserin in an attempt to improve efficacy will not improve effectiveness but may increase the risk of adverse drug effects of ketanserin, the most notorious of which being QT-interval prolongation.

Pharmacodynamic aspects

Since our pharmacokinetic analysis did not account for the lack of efficacy, we searched for a pharmacodynamic explanation, studying in vitro serotonin receptor functionality in both maternal and foetal pre-eclamptic blood vessels in comparison to vessels of normotensive pregnant women. We confirmed the presence of both 5-HT_{2A} receptors and 5-$HT_{1B/1D}$ receptors in the maternal vessels but we did - on average - not observe differences in functionality between pre-eclamptic women or normotensive women. This does not support the theory of a prominent role for 5-HT_{2A} receptors in pre-eclampsia. Our results do suggest an enhanced development of foetal 5-$HT_{1B/1D}$ receptors in pre-eclamptic patients at an earlier gestational age as compared to normotensive women. It can be speculated that in the future these observed differences can be used to study the role of selective 5-$HT_{1B/1D}$ receptors antagonists in improving the haemodynamics in umbilical artery vasculature in pre-eclamptic patients. Given the fact that the impaired uteroplacental perfusion in pre-eclamptic patients adversely affect foetal development, improvement of the umbilico-placental circulation with the use of selective 5-$HT_{1B/1D}$ receptors antagonists, may provide an additional potential for foetal growth and development.

Polymorphism

Another possibility to explain differences in drug-responses may be polymorphisms in the 5-HT_{2A} receptor. However, although 5-HT_{2A} receptor polymorphisms have been identified [5], these polymorphisms are silent and their functional significance remain obscure. Polymorphisms with respect to the metabolic pathway for ketanserin are not described in literature to our knowledge and our own findings of therapeutic plasma levels of ketanserin in all pre-eclamptic patients do not support the theory of a relevant contribution of a genetically altered metabolism of ketanserin in clinical practice.

Therefore, we did not further explore the possible involvement of polymorphisms in explaining differences in response after ketanserin administration.

Therapeutic studies in pregnant women

The lack of efficacy of ketanserin in the treatment of pre-eclampsia, stimulated the search for alternative drugs. However, pharmacotherapeutic research in pregnant women is difficult, because not only effects on the mother have to be taken into account but also effects on the foetus as well as the neonate. In the latter, both peri- and postnatal effects as well as long term effects on growth and development should be studied when analysing the safety of maternal drug use. Pharmacotherapeutic research in pre-eclamptic patients is even more difficult as the need for urgent treatment may yield several practical and ethical problems in adhering to a strict study protocol. The large differences in severity of the disease and variations in gestational age at presentation can cause methodological problems and will influence clinical decision-making. Finally, the limited placental reserve in most severe pre-eclamptic patients, the presence of a usually growth retarded foetus and the delivery of an often premature neonate who may be more susceptible to adverse drug effects, are factors that contribute to the wariness of obstetricians in testing new strategies.

For these reasons, we started our search into alternative antihypertensive drugs in patients with failure on first-line treatment, whose only remaining treatment option would have been delivery.

Nicardipine

Although information regarding the use of nicardipine in pre-eclampsia is limited, we selected this calcium-channel blocking agent because its analogue nifedipine has been used widely in pregnant women both as tocolytical drug and as oral antihypertensive drug, with satisfying outcome [6,7]. For use in pre-eclampsia, nicardipine, as compared to nifedipine, has the advantages of a more controllable effect, the availability of a parenteral formulation and less myocardial side-effects. A disadvantage can be considered the need for intravenous administration by a central venous line because of the risk on phlebitis.

Our results showing a high efficacy of nicardipine in heavily pre-treated patients, indicate that nicardipine seems to be a promising drug for treatment of pre-eclampsia. However,

some aspects still needs to be addressed. The current dosage schedule needs to be further evaluated by PK/PD studies and optimised to prevent the unwanted periods of maternal hypotension, which may lead to foetal distress. The role of plasma volume expansion in combination with use of nicardipine needs to be studied.

After establishing the optimal dosage schedule, its efficacy and safety needs to be confirmed as first-line treatment in a randomised controlled study. To obtain an adequately powered study and to allow for inclusion of women at different gestational ages, a collaboration of different hospitals at both secondary and tertiary care, will be necessary. Outcome of such a study should include foetal and neonatal safety, both short as long term. The selection of the comparison drug will however be difficult. The golden standard drug (di)hydralazin will not be available anymore on a regular basis in the Netherlands as well as in most of Europe. Our own results, described in this thesis, indicate that ketanserin is not effective in a substantial part of our patients. Probably the best comparison drug for studies evaluating nicardipine will be parenteral labetalol, but high dosages should be avoided because of case-reports of neonatal bradycardia, hypotension and hypoglycaemia.

Combination of antihypertensive drugs

Since pre-eclampsia is a progressive disease, increased antihypertensive treatment may be necessary to maintain adequate blood pressure control during treatment. Combinations of antihypertensive drugs may be more effective and safe than increasing the dosage of one drug only. When combining antihypertensive drugs, the different mechanisms of action and different side effects of the drugs can be advantageous, e.g. the opposite effects on heart rate of methyldopa in combination with nicardipine. However, in selecting consecutive treatment strategies, attention should be paid to avoid synergistically occurring side effects and to prevent unwanted hypotensive periods following cumulative blood pressure lowering effects.

Foetal and neonatal safety

Vasoactive effects on the umbilico-placental circulation after maternal antihypertensive drug use, can influence foetal safety, in relation to the maternal haemodynamic changes. Studies using *ex vivo* placental perfusion may be helpful in determining effects of these drugs on the foetoplacental vascular bed.

On the other hand, specific pharmacological effects of the drug can also occur because of placental drug transfer. Management of pre-eclampsia with parenteral antihypertensive drugs is usually limited to 2 - 3 weeks prior to delivery, albeit in high dosages. Following case-reports of neonatal bradycardia, hypotension and hypoglycaemia after maternal use of labetalol, it became recognised that transplacental transmission can occur after antihypertensive treatment of pre-eclampsia, resulting in adverse neonatal effects. In this thesis, we studied transplacental transmission of ketanserin and nicardipine, showing a

high transplacental transmission of ketanserin with subsequent high neonatal blood levels, whereas a relative low transmission of nicardipine was found.

Furthermore, our results of an intermediate milk/plasma ratio for ketanserin as opposed to a low milk/plasma ratio for nicardipine in breastmilk suggest that for prolonged (oral) antihypertensive treatment in lactating women after delivery, nicardipine is preferred to ketanserin.

The level of placental transmission, determined by the umbilical cord-maternal blood ratio at delivery, is useful to indicate the level of foetal exposure. However, the ultimate goal is to establish whether adverse effects in the neonate occur because of maternal drug use. Since serotonin receptors are known to develop extensively early in pregnancy in the foetal brain, a possible influence on the functionality of foetal serotonin receptors by maternal ketanserin treatment is theoretically possible and may even have long term consequences for the child. Fortunately, our *ex vivo* data studying foetal 5-HT receptor functionality after maternal ketanserin use could not confirm such an influence.

For nicardipine, the lack of adverse effects on neonatal well-being after maternal drug use during pregnancy, still needs to be confirmed in larger comparative studies, preferably including a long term follow-up of the neonates. The current policy in our hospital of a regular follow-up of the neonates into their childhood, after discharge from our neonatal intensive care unit, will contribute to the analysis of the safety of nicardipine.

Future perspectives

In the future, with the unravelling of the pathogenesis of pre-eclampsia, pharmacological interventions to prevent pre-eclampsia or to treat the disorder more specifically, instead of ameliorating only the symptoms, may become feasible. During several decades, the pathophysiology of pre-eclampsia has been extensively studied. The recent discovery of the involvement of an imbalance among the pro- and antiangiogenic factors in the failure of placental vasculogenesis could be of significance [8]. The antiangiogenic protein sFlt1 (soluble fms-like tyrosine kinase) was found to be up-regulated in the placenta of women in conjunction with decreased VEGF (vascular endothelial growth factor) and decreased PlGF (placental growth factor). These alterations in angiogenic balance are hypothesized to contribute to placental ischemia and maternal endothelial dysfunction in pre-eclampsia. If the levels of these proteins are found to be specific predictors of pre-eclampsia early in pregnancy, interventions can be undertaken before extensive vascular dysfunction and severe maternal complications occur or before severe intra-uterine growth retardation due to placental dysfunction has occurred. Indeed, if sFlt-1 is confirmed to be a causal factor in the pathophysiology of pre-clampsia, pharmacological agents counteracting the effect of sFlt-1, can be of clinical importance in treatment of pre-eclampsia.

At this time, supportive treatment using antihypertensive drugs is the only available treatment option for pre-eclampsia (except for delivery). Use of antihypertensive drugs should be targeted at the safe treatment of the mother while at the same time improving perinatal outcome. To avoid the current situation that well studied drugs are not available anymore and drugs that have not been adequately studied have to be used in this population of high-risk patients, pharmacological research into antihypertensive treatment of pre-eclampsia is needed.

This thesis has focused on the efficacy and safety of two new antihypertensive drugs, the serotonin antagonist ***ketanserin*** and the calcium-channel blocking agent ***nicardipine***. Our data show that administration of ketanserin results in insufficient antihypertensive response in many severe pre-eclamptic patients and that increasing the dosage will not solve this problem. Nicardipine appears to be a potent alternative drug with the advantage of a low placental transfer and it may become a standard treatment modality in pre-eclampsia.

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Summary and conclusions

Pre-eclampsia occurs in 2 – 7% of the pregnancies and the disorder still forms the leading cause of maternal and neonatal mortality and morbidity in pregnancy in the Netherlands. Pre-eclampsia is characterised by the occurrence of elevated blood pressure and proteinuria after the 20th week of pregnancy, which may lead to serious maternal complications, such as HELLP-syndrome (haemolysis, elevated liver enzymes, low platelet counts), abruptio placentae and eclampsia. Increased perinatal morbidity and mortality may occur, following intra-uterine growth restriction, reduced amniotic fluid and abnormal oxygenation. The pathogenesis remains unknown but most hypothesis focus on vascular dysfunction and maternal-foetal (paternal) immune maladaptation.

The high maternal blood pressure in severe pre-eclampsia poses a serious risk of acute cerebrovascular complications in these women, necessitating the use of potent antihypertensive treatment in pre-eclampsia. However, the availability of parenteral antihypertensive drugs for use in pre-eclampsia has decreased in the past years. Also, a controversy has risen surrounding the risks and benefits of the current standard drug (di)hydralazin.

In this thesis, the efficacy and safety in pre-eclampsia of two new antihypertensive drugs, the 5-HT_{2A} antagonist ***ketanserin*** and the calcium-channel blocking agent ***nicardipine***, are studied.

In **CHAPTER 1** the different antihypertensive drugs, used in the management of pre-eclampsia, are reviewed with a focus on efficacy and maternal and neonatal safety. The characteristics of each drug are summarized, including placental transfer. Comparisons between antihypertensive drugs are described, based on recent publications.

The 5-HT_{2A} receptor antagonist the drug ***ketanserin*** is one of the two drugs licensed in the Netherlands for use in pre-eclampsia and has been used as first-line parenteral treatment in many Dutch hospitals. The drug acts by blocking the vasoconstrictive response upon binding of serotonin (5-HT) to 5-HT_{2A} receptors in vascular tissue. Its use is based upon the concept that pre-eclampsia is characterised by endothelial dysfunction. Due to the concomitantly loss of 5-HT_1 endothelial receptors, free serotonin will stimulate mainly 5-HT_{2A} receptors in platelets causing platelet aggregation and serotonin release, and 5-HT_{2A} receptors in vascular smooth muscle, causing vasoconstriction, which contributes to the elevated blood pressure.

Because of ambiguous reports on the efficacy of ketanserin in pre-eclampsia, we analysed in **CHAPTER 2** retrospectively the efficacy of intravenous ketanserin in controlling blood pressure of a population of severe early-onset pre-eclamptic patients. Pre-eclamptic patients (n = 47) with a gestational age between 21 and 32 weeks were treated with intravenous ketanserin at a maximum dosage of 14 mg/h, to obtain an intra-arterial diastolic blood pressure of 90 mmHg or below. We found that, even using the maximum intravenous dosage

of ketanserin, target blood pressure was not achieved in 15 (32%) patients. A high systolic blood pressure at the start of treatment was associated with failure of ketanserin treatment. The median period of ketanserin treatment in the responding group was three days (range 1 - 10 days). In 26 (55%) of initially successfully treated patients, additional antihypertensive drugs were necessary to maintain blood pressure control.

It was concluded that administration of intravenous ketanserin does not result in an adequate antihypertensive response in a substantial proportion of severe pre-eclamptic patients, despite high dosages. These results lead us to investigate in further detail the insufficient response on ketanserin.

To perform a pharmacokinetic analysis, a sensitive and selective high-performance liquid chromatographic assay with solid-phase extraction and fluorimetric detection was developed and validated for the quantification of ketanserin and its metabolite ketanserinol in human plasma, as described in **CHAPTER 3**. Calibration curves were linear in the range of 0 – 400 ng/ml for both ketanserin and ketanserinol and the lower limit of quantification for both ketanserin and ketanserinol was 2.0 ng/ml. The small sample size required in this assay made the method useful for determination of drug concentrations in neonates.

As described in **CHAPTER 4**, plasma concentrations of ketanserin were determined in 51 pre-eclamptic patients. Population pharmacokinetic parameters were assessed, using the iterative two-stage Bayesian population procedure. The influence of individual pharmacokinetic parameters on antihypertensive response, expressed as the attainment of a diastolic blood pressure ≤ 90 mmHg using ketanserin treatment, was analyzed.

Almost all plasma concentrations of ketanserin were found to be in or even above the therapeutic range, indicating that the current dosage schedule results in pharmacologically active plasma concentrations. The individual pharmacokinetics of ketanserin in pre-eclamptic patients showed an accurate fit, applying a three-compartment model. A metabolic clearance (Cl_m) of 37.9 ± 10.86 L/h and a volume of distribution (V1) of 0.544 ± 0.188 L/kg were found in the pre-eclamptic patients, which is comparable with historical data from healthy volunteers. Despite a considerable interindividual variation, no correlation was found between differences in Cl_m or V1 and antihypertensive response, indicating that variation in the pharmacokinetic parameters does not explain the lack of response in pre-eclamptic patients.

Subsequently, the role of pharmacodynamics was studied in the search for an explanation for the limited efficacy of ketanserin.

In **CHAPTER 5**, we describe the comparison of the functional reactivity of 5-HT receptors in foetal and maternal blood vessels in pre-eclamptic pregnancies and normotensive pregnancies. Foetal umbilical cord arteries were used as representative of foetal blood vessels, and subcutaneous fat arteries were used as representative of maternal resistance

arteries. Segments of arteries were mounted in tissue baths and isometric force changes were determined after exposure to 5-HT and the 5-$HT_{1B/1D}$ receptor agonist, sumatriptan. Concentration-response curves to 5-HT and sumatriptan were constructed in the absence and presence of ketanserin or GR125743 (5-$HT_{1B/1D}$ receptor antagonist). We found that both 5-HT and sumatriptan contracted all segments studied, confirming that 5-$HT_{1B/1D}$ and 5-HT_{2A} receptors mediate vasoconstriction in these arteries. The responses to 5-HT and the potency of ketanserin in umbilical cord arteries were not different between the study groups, indicating similar characteristics of the foetal 5-HT_{2A} receptor in pre-eclampsia and normotensive pregnancies. In contrast, the potencies of both sumatriptan and GR125743 in umbilical cord arteries were positively correlated to gestational age in the normotensive group, while this relationship was absent in the pre-eclamptic group. We concluded that the sensitivity of foetal 5-$HT_{1B/1D}$ receptors increases in the last trimester in normal pregnancies, which seems to be expedited in pre-eclamptic patients.

In maternal resistance arteries, responses to 5-HT and sumatriptan were not different between the pre-eclamptics patients and normotensive controls. These findings do not support the theory of a prominent role for 5-HT_{2A} receptors in pre-eclampsia and may explain the disappointing efficacy of a 5-HT_{2A} receptor blocking agent, such as ketanserin.

Foetal and neonatal safety are important issues to address when evaluating maternal drug use during pregnancy. For ketanserin, no apparent clinical adverse effects were seen in neonates after maternal drug use, but detailed information regarding transplacental transfer and transmission in breast milk was not available.

In **CHAPTER 6**, a prospective, observational study is described, assessing transplacental transfer of ketanserin, transfer into breast milk and disposition in the neonate after maternal ketanserin use. In 22 pregnant women with severe pre-eclampsia, the ratio of drug concentrations in the umbilical cord to drug concentrations in maternal blood just before delivery was used as a indicator of placental transfer. Disposition of ketanserin was determined using neonatal plasma concentrations of ketanserin in eight neonates after birth.

A high placental transfer was found in the pre-eclamptic women (median transfer 0.95, range 0.612-1.24) for ketanserin and for its metabolite, ketanserinol (median transfer 0.60, range 0.5-0.77). Pharmacologically active concentrations of ketanserin were found in the neonate after delivery. The elimination half-life of ketanserin in the neonate varied between 12.7 and 43.7 h (median 19.3 h) and of ketanserinol between 13.8 and 34.4 h (median 18.7 h). Despite the high placental transfer and the substantial disposition in the neonate, no apparent short-term adverse effects in the neonates were detected.

A median breast milk/ plasma ratio for ketanserin of 0.4 (range, 0.2 – 1.27) and 0.76 (range, 0.42 – 1.44) for ketanserinol, was found after analysing breast milk samples and corresponding maternal plasma concentrations of seven lactating women during use of

intravenous ketanserin. This indicates that the neonate may be exposed to pharmacological concentrations of ketanserin and ketanserinol during lactation.

Given the abundant presence of 5-HT receptors in the foetus, including the foetal brain, more safety data on the influence of the exposure to ketanserin on foetal 5-HT receptors after maternal use, were needed. Functional studies were performed on 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors in umbilical cord artery from pre-eclamptic patients treated with ketanserin (**CHAPTER 7**). Umbilical cord arteries were obtained immediately after delivery from pre-eclamptic patients (n=7, gestational age 28 weeks, range 25^{4} – 36^{3} weeks), treated antenatally with intravenous ketanserin. Pre-eclamptic patients (n=13, GA 29^{4} weeks, range 24^{4} – 36^{6} weeks), not treated with ketanserin, were included as a control group. Segments of umbilical cord arteries were mounted in tissue baths and isometric force changes were measured. Cumulative concentration-response curves to 5-HT and to the 5-$HT_{1B/1D}$ receptor agonist, sumatriptan, were constructed in the absence or presence of the 5-HT_{2A} receptor antagonist ketanserin or the 5-$HT_{1B/1D}$ receptor antagonist GR125743, respectively.

All segments showed contractile response to both 5-HT and sumatriptan, and the concentration-response curves showed a rightward shift with increasing concentrations of ketanserin and GR125743, respectively, indicating the presence of functional 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors in the foetal tissue. No significant differences were found in maximum response or potency of 5-HT in both groups and no significant differences were found in the potency of the antagonist ketanserin in both study groups (pK_b 7.58 ± 0.37 in ketanserin treated group and 7.46 ± 0.17 in the control group, respectively). Similarly, with sumatriptan, no significant differences were found between ketanserin-treated patients and control patients.

We concluded that foetal exposure to ketanserin in pre-eclamptic patients does not influence the functional characteristics of 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors in the umbilical cord artery.

The insufficient response on ketanserin in the pre-eclamptic patients stimulated the search for alternative drugs.

The calcium-channel blocking agent ***nicardipine*** was selected for further study because of its controllable antihypertensive effect due to the short half-life, the availability of a parenteral formulation and the advantage of less myocardial side-effects compared to its analogue nifedipine, which is used as tocolytical drug and as oral antihypertensive drug in pre-eclampsia.

Because data on efficacy and safety of nicardipine in pre-eclampsia were scarce, we started to evaluate prospectively the efficacy of intravenous administration of nicardipine as a second-line temporizing treatment in severe, early-onset, pre-eclamptic patients (**CHAPTER 8**). Nicardipine was administered to 27 early-onset, pre-eclamptic patients with a median gestational age of 27 weeks (range 21 – 32 weeks) with treatment failure on standard

intravenous antihypertensive drugs (ketanserin, dihydralazin or labetalol). Nicardipine infusion was initiated at a dosage of 3 mg/h and was titrated according to blood pressure. Treatment was continued for as long as the maternal and foetal conditions allowed. In all patients the target diastolic intra-arterial blood pressure (<100 mmHg or <90 mmHg in HELLP-syndrome patients), registered by continuous arterial blood pressure measurements, was obtained within a median of 23 min (range, 5 – 60 min). Delivery was postponed for a median of 4.7 days (range, 1 – 26 days) using nicardipine treatment, at a maximum dosage ranging from 3 to 9 mg/h. In 30% of the patients, unwanted hypotensive periods (< 70 mmHg) were registered during treatment, manageable with dosage adaptation. Foetal well-being did not seem adversely affected.

This evaluation showed that nicardipine is a potent antihypertensive drug, even in severe, early-onset pre-eclampsia when other antihypertensive drugs have failed. The dosage-schedule needs to be optimized to prevent maternal hypotension.

The safety of nicardipine in pre-eclampsia was further analyzed by studying the transplacental transfer and disposition in human milk (**CHAPTER 9**).

In ten pre-eclamptic patients, nicardipine concentrations of maternal blood and both arterial and venous umbilical cord blood samples were assessed and the ratio of maternal (P) and foetal plasma concentrations ($U_{arterial}$ and U_{venous}) was calculated as an indication of transplacental transfer. We found a median transfer of 0.15 ($U_{arterial}$/P, range 0.05-0.22), and 0.17 (U_{venous}/P, range 0.023 – 0.22). The concentration nicardipine in umbilical cord correlated with the maternal nicardipine dosage at delivery. Umbilical cord concentrations ranged between 1.3 and 18 ng/ml, which can be considered as subtherapeutic.

Nicardipine levels were determined in 34 breast milk samples of seven patients and in 80% of the samples, nicardipine was below the lower limit of detection of 5 ng/ml. The maximum possible exposition of a neonate to nicardipine was extrapolated to be 300 ng/day, which is insignificant compared to therapeutic dosages used in neonates.

We concluded that the exposition of a foetus and neonate to nicardipine by transplacental transmission and disposition in breast milk is low.

In **CHAPTER 10** our results are discussed and future perspectives are indicated. We conclude that administration of ketanserin results in insufficient antihypertensive response in a considerable number of severe pre-eclamptic patients and that increasing the dosage probably does not solve this problem.

Nicardipine appears to be a potent alternative drug with the advantage of a low placental transfer, as opposed to the extensive placental transfer found for ketanserin. Further experience in the use of nicardipine as first-line treatment in pre-eclampsia is needed, including more data on the optimal dosage schedule and more data on neonatal safety, to confirm the promising role of nicardipine in pre-eclampsia.

APPENDICES

SAMENVATTING

Bij 2 - 8% van de zwangere vrouwen treedt zwangerschapsvergiftiging (pre-eclampsie) op tijdens de zwangerschap of het kraambed. De aandoening kenmerkt zich door een plotseling sterk stijgende bloeddruk, eiwit in de urine en vochtophoping in enkels, handen en gezicht en klachten van hoofdpijn, misselijkheid en slecht kunnen zien. Zwangerschapsvergiftiging kan zowel de moeder als het kind in gevaar brengen. Bij de moeder kunnen als gevolg van de sterk verhoogde bloeddruk, ernstige complicaties ontstaan, zoals het HELLP-syndroom (gekenmerkt door afbraak van rode bloedcellen, verhoogde leverenzymen en een verminderd aantal bloedplaatjes), epileptische aanvallen (eclampsie), lever- en nierbeschadiging, loslating van de placenta, of een hersenbloeding. Bij het kind kan door een verminderde aanvoer van voedingsstoffen groeiachterstand optreden en door langdurig zuurstoftekort kunnen hersenbeschadigingen, geboorteafwijkingen en zelfs overlijden van het ongeboren kind het gevolg zijn. Veelal moeten de kinderen vroegtijdig met een keizersnede ter wereld worden gebracht.

Een stoornis in de aanleg van de placenta wordt gezien als de belangrijkste factor in het ontstaan van pre-eclampsie. Een goede doorbloeding van de placenta wordt bij een ongestoorde zwangerschap gewaarborgd doordat na innesteling van de vrucht in de baarmoeder, cellen van het embryo ingroeien in de bekleding van de bloedvaten van de moeder. Hierdoor gaan deze bloedvaten van de moeder sterk verslappen en daarmee wijd open staan om zodoende voldoende bloed met voedingsstoffen en zuurstof door te kunnen laten tijdens de zwangerschap. Men veronderstelt dat bij pre-eclampsie deze ingroei van cellen van het embryo in de bloedvaten van de moeder niet goed plaatsvindt. De oorzaak van pre-eclampsie is niet volledig opgehelderd, maar genetische, immunologische en mogelijk ook omgevingsfactoren lijken een rol te spelen.

Tot op heden is de enige manier om pre-eclampsie te verhelpen, het beëindigen van de zwangerschap. Echter, wanneer pre-eclampsie vroeg in de zwangerschap optreedt, kan de vroeggeboorte van het kind ernstige complicaties voor het kind met zich mee brengen en zelfs diens leven bedreigen. Daarom wordt in die gevallen geprobeerd de zwangerschap te verlengen door het toedienen van bloeddrukverlagende middelen aan de moeder. Het probleem hierbij is dat er slechts zeer weinig bloeddrukverlagende middelen hiervoor gebruikt kunnen worden. De beschikbaarheid van het meest toegepaste bloeddrukverlagend middel dihydralazine is in Nederland inmiddels sterk afgenomen, zodat onderzoek naar alternatieve bloeddrukverlagende middelen noodzakelijk is.

Het onderzoek, beschreven in dit proefschrift, gaat over de effectiviteit en veiligheid van twee nieuwe bloeddrukverlagende middelen bij de behandeling van zwangerschaps-vergiftiging, namelijk de serotonine receptor antagonist ***ketanserine*** en de calcium-instroom blokker ***nicardipine.*** De eigenschappen van beide geneesmiddelen worden hieronder verder beschreven.

In **Hoofdstuk 1** worden de verschillende geneesmiddelen die worden toegepast bij de behandeling van pre-eclampsie besproken. Hoewel veel onderzoek is gedaan naar geneesmiddelen die het ontstaan van pre-eclampsie zouden kunnen voorkómen, is er helaas tot nu toe geen doeltreffend middel hiervoor gevonden. Wanneer een patiënt wordt opgenomen met symptomen van pre-eclampsie, is het toedienen van bloeddrukverlagende medicatie de enige behandelingsmogelijkheid, indien uitstel van de bevalling is gewenst vanwege het onvoldragen kind. Zowel oraal als intraveneus toegediende bloeddrukverlagende geneesmiddelen worden toegepast. In dit hoofdstuk wordt, op basis van recente publicaties, ingegaan op de effectiviteit en eventuele bijwerkingen voor de moeder van de verschillende geneesmiddelen. Ook wordt de mogelijke invloed van de geneesmiddelen op de veiligheid van het (ongeboren) kind besproken.

(Di)hydralazine is het meest gebruikte bloeddrukverlagend geneesmiddel bij de behandeling van ernstige pre-eclampsie. Na intraveneuze toediening werkt het direct op de bloedvaten, waardoor verwijding van de vaten optreedt en de bloeddruk van de moeder daalt. Het geneesmiddel is effectief maar geeft wel vaak bijwerkingen bij de moeder, zoals hoofdpijn en misselijkheid. Recent is uit een grote analyse gebleken dat bij gebruik van (di)hydralazine mogelijk meer nadelige effecten bij het ongeboren kind zouden optreden dan bij andere middelen. Een belangrijke factor hierbij is de dosis en de wijze van toediening van (di)hydralazine, waarbij een té lage bloeddruk bij de moeder voorkomen moet worden, omdat de doorbloeding van de placenta mogelijk in gevaar kan komen.

Ketanserine is een bloeddrukverlagend middel dat zijn werking ontleent aan een blokkade van de zogeheten 5-HT_{2A} receptoren. De toepassing bij pre-eclampsie berust op het feit dat wordt aangenomen dat de wand van bloedvaatjes is beschadigd bij een pre-eclamptische patiënt. Hierdoor kan in het bloed aanwezig serotonine (5-HT) binden aan 5-HT_{2A} receptoren, die zich onder de beschadigde wand van het bloedvat bevinden. Dit heeft een bloedvatvernauwing tot gevolg waardoor de bloeddruk van de moeder stijgt. Ketanserine voorkomt dat serotonine zich bindt aan de 5-HT_{2A} receptor waardoor minder bloedvatvernauwing zal optreden.

In **Hoofdstuk 2** wordt de effectiviteit en veiligheid van het gebruik van ketanserine bij 47 patiënten met ernstige pre-eclampsie beschreven, met een zwangerschapsduur tussen 21 en 32 weken bij start van de behandeling. De dosering van ketanserine werd aangepast op geleide van de bloeddruk tot een maximale dosis van 14 mg/uur intraveneus. Helaas bleek het middel bij 32% van de patiënten niet in staat om de bloeddruk voldoende te laten dalen, ondanks maximale doseringen. Een hoge systolische bloeddruk bij start van de behandeling bleek geassocieerd te zijn met onvoldoende reactie op ketanserine. De zwangerschap kon in de groep patiënten die aanvankelijk goed reageerden met 3 dagen worden verlengd (range 1-10 dagen). Bij 55% van deze patiënten was gedurende de behandeling toevoeging van andere bloeddrukverlagende middelen noodzakelijk om de bloeddruk onder controle te

houden. Hieruit werd geconcludeerd dat de effectiviteit van ketanserine bij een aanzienlijk deel van de vroege, ernstige pre-eclamptische patiënten onvoldoende is.

Een van de oorzaken van de onvoldoende effectiviteit kan zijn dat de concentratie van ketanserine in het bloed van een pre-eclamptische patiënt te laag is voor een adequate respons. Om dit te kunnen onderzoeken, werd een chromatografische methode opgezet en gevalideerd voor de bepaling van ketanserine en zijn metaboliet ketanserinol in plasma (**Hoofdstuk 3).**

Bij 51 pre-eclamptische patiënten werd de concentratie van ketanserine in plasma bepaald en werden farmacokinetische parameters berekend. Uit dit onderzoek, beschreven in **Hoofdstuk 4,** bleek dat in vrijwel alle patiënten de concentratie van ketanserine in het bloed voldoende hoog was om een bloeddrukverlagend effect te mogen verwachten. De gevonden klaring (Cl_m 37.9 ± 10.86 L/uur) en het verdelingsvolume (V1 0.544 ± 0.188 L/kg) waren vergelijkbaar met historische waardes van gezonde vrijwilligers. Er werd geen correlatie gevonden tussen verschillen in klaring en verdelingsvolume en een adequate respons op ketanserine. Dit maakt het onwaarschijnlijk dat variaties in de farmacokinetiek de oorzaak zijn van de beperkte effectiviteit van ketanserine.

Vervolgens is onderzocht of een verschil in de functionaliteit van serotonine receptoren in de bloedvaten van pre-eclamptische patiënten een verklaring zou kunnen vormen voor de verminderde effectiviteit van ketanserine (**Hoofdstuk 5)**. Zowel bij pre-eclamptische zwangeren als bij zwangeren zonder pre-eclampsie (controle groep) werden bloedvaatjes uit het onderhuids vetweefsel van de moeder en van de navelstreng (representatief voor foetaal weefsel) ex vivo onderzocht. De vaatjes werden blootgesteld aan serotonine en aan sumatriptan (een verbinding die selectief aangrijpt op $5\text{-HT}_{1B/D}$ receptoren). De contractie werd gemeten, zowel in afwezigheid als in aanwezigheid van opklimmende concentraties van specifieke $5\text{-HT}_{1B/D}$ en 5-HT_{2A} receptor blokkerende stoffen. Uit dit onderzoek bleek dat zowel $5\text{-HT}_{1B/D}$ als ook 5-HT_{2A} receptoren betrokken zijn bij vernauwing van deze bloedvaten. Er bleek geen verschil aantoonbaar tussen de pre-eclamptische patiënten en de controle groep wat betreft functionaliteit van 5-HT_{2A} receptoren in de navelstreng en de bloedvaten van de moeder. Hiermee lijken 5-HT_{2A} receptoren een ondergeschikte rol te spelen in de onderliggende oorzaken van pre-eclampsie, hetgeen een verklaring kan zijn voor de beperkte effectiviteit van ketanserine.

Voor $5\text{-HT}_{1B/D}$ receptoren werd in foetaal weefsel van de controle groep aangetoond dat de zwangerschapsduur van invloed is op de ontwikkeling van deze receptoren. Deze invloed kon niet worden aangetoond bij pre-eclamptische zwangeren. Bij deze patiënten waren de $5\text{-HT}_{1B/D}$ receptoren al vroeg in de zwangerschap volledig functioneel.

Om de veiligheid van het gebruik van ketanserine in de zwangerschap beter te kunnen beoordelen, werd gekeken naar de overgang van ketanserine door de placenta naar het bloed van de foetus en naar de mate waarin ketanserine voorkomt in het bloed van de pasgeborene (**Hoofdstuk 6**). Bij 22 pre-eclamptische zwangeren die ketanserine gebruikten, werd de verhouding tussen de concentratie in navelstrengbloed en de concentratie in het bloed van de moeder vlak voor de bevalling, gebruikt als maat voor de placentaire overgang van ketanserine. Het bleek dat ketanserine de placenta nagenoeg volledig passeerde (gemiddelde verhouding concentratie navelstrengbloed/concentratie moeder: 0.95 [range 0.6-1.24]). Farmacologisch actieve concentraties van ketanserine werden aangetoond in het bloed van pasgeborenen. Ook werden na de bevalling ketanserine concentraties bepaald in moedermelk van zeven patiënten. Ketanserine bleek in redelijke mate over te gaan in moedermelk (gemiddelde verhouding moedermelk/plasmaconcentratie van 0.4 [range 0.2-1.27]). Hoewel er klinisch geen aanwijsbare bijwerkingen bij de foetus of pasgeborene werden gevonden, geven de resultaten van dit onderzoek aan dat effecten bij het kind kunnen optreden ten gevolge van gebruik van ketanserine bij de moeder.

Serotonine receptoren worden al vroeg in de zwangerschap bij de foetus aangelegd, met name in de hersenen van het kind. In **Hoofdstuk 7** is het onderzoek beschreven waarin is nagegaan of de blootstelling van de foetus aan ketanserine via de moeder nadelige effecten heeft op de ontwikkeling van foetale serotonine receptoren. Hiertoe werd de functionaliteit van 5-$HT_{1B/D}$ en 5-HT_{2A} receptoren uit navelstrengvaten vergeleken (op de wijze zoals beschreven in Hoofdstuk 5) tussen zeven vroege, pre-eclamptische zwangeren die ketanserine hadden gebruikt en dertien pre-eclamptische zwangeren die geen ketanserine hadden gebruikt, met eenzelfde zwangerschapstermijn. Er werden geen verschillen gevonden tussen de twee groepen wat betreft functionaliteit van beide receptortypes, waardoor ketanserine in dat opzicht als relatief veilig voor de foetus kan worden beschouwd.

Vanwege de beperkte effectiviteit van ketanserine bij de ernstige pre-eclampsie patiënten, werd onderzoek verricht naar een geschikt alternatief bloeddrukverlagend middel. De calcium-instroom blokker ***nicardipine*** werd geselecteerd omdat naar verwachting, de snelle werking en de korte werkingsduur na intraveneuze toediening een goed controleerbare bloeddrukregulatie mogelijk maakt. Het bloeddrukverlagende effect berust op het blokkeren van de instroom van calcium-ionen in specifieke kanaaltjes in het bloedvat, waardoor verwijding van de bloedvaten optreedt. Hoewel nicardipine niet is geregistreerd voor gebruik tijdens de zwangerschap, worden de risico's gering geacht, omdat het hieraan verwante middel, nifedipine, al eerder veilig is gebleken voor het ongeboren kind bij toepassing als weeënremmer en als oraal bloeddrukverlagend middel tijdens de zwangerschap.

In **Hoofdstuk 8** staan de resultaten beschreven van de toepassing van intraveneus nicardipine als tweedelijns behandeling bij 27 pre-eclampsie patiënten, waarbij ketanserine of dihydralazine onvoldoende effectief was of tot onaanvaardbare bijwerkingen leidde. Nicardipine werd gestart in een dosis van 3 mg/uur en opgehoogd op geleide van de bloeddruk tot maximaal 9 mg/uur. Binnen 20 minuten werd de streefwaarde voor de diastolische bloeddruk (< 100 mmHg) bereikt in alle patiënten. De zwangerschap kon met gemiddeld 4.6 dagen (range 1 – 26 dagen) worden verlengd. Bij een derde van de patiënten daalde de bloeddruk echter incidenteel tot te lage waardes (< 70 mmHg), waarop de dosis moest worden verlaagd. Er werd geen relatie tussen de hypotensieve periodes en het optreden van foetale nood vastgesteld.

Geconcludeerd werd dat nicardipine een potent bloeddrukverlagend middel is, dat goed bruikbaar is als tweedelijns behandeling van pre-eclampsie. Gezien het frequent optreden van periodes met een té lage bloeddruk, dient het doseerschema nog verder te worden geoptimaliseerd.

Ook voor nicardipine werd de overgang door de placenta en naar moedermelk bepaald (**Hoofdstuk 9)**. In tien pre-eclamptische patiënten werd uitgaande van de bloedconcentraties in de navelstreng ten opzichte van de concentraties in het bloed van de moeder, een placentaire overgang gevonden van gemiddeld 20% (0.023-0.22). De gevonden bloedconcentraties in de navelstreng varieerden tussen 1.3 en 18 ng/ml, hetgeen zo laag is dat belangrijke effecten bij het (ongeboren) kind niet waarschijnlijk zijn. In 34 porties moedermelk van zeven patiënten werd nicardipine bepaald, waarbij in 80% van de porties, nicardipine niet aantoonbaar was. De maximaal mogelijke blootstelling van een pasgeborene via de moedermelk werd berekend als 300 ng/dag, hetgeen een zeer geringe hoeveelheid is. Deze resultaten geven aan dat nicardipine een gunstig profiel heeft ten aanzien van kans op effecten op het kind bij gebruik in de zwangerschap of tijdens het geven van borstvoeding.

In **Hoofdstuk 10** wordt geconcludeerd uit de onderzoeken, zoals beschreven in dit proefschrift, dat ketanserine onvoldoende werkzaam is in een aanzienlijk deel van de vroege, pre-eclamptische patiënten. Gezien het feit dat bij vrijwel alle pre-eclamptische patienten hoge concentraties ketanserine in het bloed worden aangetoond en gezien het feit dat serotonine receptoren geen belangrijke rol lijken te spelen bij pre-eclampsie, kan geconcludeerd worden dat dosisverhoging van ketanserine weinig toegevoegde waarde zal hebben.

Uit de onderzoeken waarin intraveneus nicardipine is toegepast, blijkt een goede effectiviteit bij vroege pre-eclamptische patiënten en een geringe overgang door de placenta. Verder onderzoek moet zich richten op het ontwikkelen van een optimaal doseerschema en op bevestiging van de gunstige werkzaamheid en veiligheid van nicardipine als eerste-lijns behandeling, ten opzichte van andere bloeddrukverlagende middelen.

Geconcludeerd wordt dat intraveneus nicardipine een veelbelovend bloeddrukverlagend geneesmiddel is bij de behandeling van pre-eclampsie.

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LIST OF PUBLICATIONS RELATED TO THIS THESIS

Hanff LM, Visser W, Vulto AG, Steegers EAP (2006). Pharmacological management of severe pre-eclampsia. European Clinics in Obstetrics and Gynaecology. In press

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ABOUT THE AUTHOR

Lidwien Hanff was born on the 3rd of October, 1965 in Venlo, the Netherlands. After Grammar-school at the Marianum in Venlo, she studied Pharmacy at the University of Leiden and the University of Groningen. During her study, she performed a research project on toxicology at the Poisons Unit, St. Guy's Hospital in London.

She obtained her pharmaceutical degree in 1990, and started as a pharmacist in the Drechtsteden Hospital in Dordrecht (head: Drs. R.M. Posthuma). From 1991 to 1994 she trained as a hospital pharmacist in the Medisch Spectrum Twente in Enschede (head: Dr. W.J.M.J. Rutten). She started in 1994 as a hospital pharmacist at the Department of Pharmacy of Erasmus Medical Center Rotterdam (head Drs. B.H. Graatsma, and subsequently Dr. P.J. Roos). From 1999 on, she is responsible for the pharmacy department of the Sophia Children's Hospital of the Erasmus Medical Center. Simultaneously, she started her research on the treatment of pre-eclampsia in close collaboration with Dr. W. Visser of the Department of Obstetrics, which forms the basis of the thesis. She has an interest in paediatric clinical pharmacy and pharmacology and spent in 2003 a period at the pharmacy department of the Children's Hospital in Boston.

Lidwien Hanff is married to Henk-Jan Guchelaar and they live in Gouda with their two sons Niels en Daan.