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**INTERACTION OF DRUGS IN THE PATHOGENESIS OF FETAL ANOMALIES  
(NEUROTOXIC EFFECT OF TARDYL® IN HUMAN FETUSES)**

Doctoral (PhD) theses



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## INTRODUCTION

The study of congenital anomalies is called human teratology. Its primary aims are to explore and prevent external harms that cause development disorders in fetuses. Development disorders may be morphological and structural (congenital abnormalities, CA), fetopathy, intrauterine growth retardation of the fetus, genetic disorders and, finally, functional disorders, e.g. mental retardation. The significance of congenital anomalies in terms of public health is partly due to the fact that they represent a state of defect from which – depending on the severity – full recovery is not possible. On the other hand, severe CAs are observable as early as at birth, and the development of such can be traced back to the time of pregnancy, primarily to months II and III. Consequently, the only obviously optimal solution would be to prevent CAs.

Out of functional disorders, in my study I have investigated mental retardation and behavioural deviations, as potential consequences of neurotoxic effects. Mental retardation (MR) affects approximately 3% of all children. It is a functional state that involves narrowed adaptive skills and measurably reduced intelligence.

## OBJECTIVES

The objective of my PhD dissertation was to investigate the potential teratogenic and/or fetotoxic effects of four drugs taken during pregnancy (amobarbital, glutethimide, promethazine and a combination of the three, Tardyl<sup>®</sup>).

1. The evaluation of clinical doses of potential teratogenic and/or fetotoxic effects of these drugs in a case-control approach.
2. To use of the self-poisoning pregnant population – as a group of subjects exposed to a one-off extremely high dose of drugs – regarding the evaluation of the teratogenicity and/or fetotoxicity of four drugs.
3. To verify whether the model of self-poisoning pregnant women based on large doses results a more efficient assessment of teratogenicity/fetotoxicity of drugs, as clinical doses in the usual epidemiological studies, thus better reflects a balance of costs and benefits of drug treatments.

On assessing **teratogenicity of drugs**, certain criteria of human teratology must be used as a starting point, of which the key factors are as follows:

1. *The time of taking the drugs:* I. In the pre-embryonic period (post-conception weeks 1 and 2) external harm is characterized by the “all or nothing” rule: the zygote either dies or keeps developing in health.
- II. Organogenesis. External harms cause most CAs in the embryonic period (post-conceptional weeks 3 to 10), because this is when the organs and bodily forms of the embryo are formed. The times of development of various CA units are different (it is called the critical period). At this time, a teratogenic effect may have three consequences: fetal death; CAs and healthy birth due to regeneration of fetal tissues and organs or a mildness of the adverse effect.
- III. In the period of growth and maturation (from post-conceptional week 10),

development of the neural system is still in progress, and this is also when the fetus' immune system starts functioning. In this period, drastic harms may have four consequences: fetal death; fetopathy (diseases of the fetus) and neurological disorders; rarely CAs and healthy birth.

2. *Specificness of teratogens*: only the drugs that cause clearly definable special CAs can be considered as teratogenic. On the other hand, the nature of CAs often lends itself to subsequent identification of the teratogen. At the same time, a wide variety of expressions is caused by the duration and dose of the exposure, the mother's and the fetus' genotypes, the gestation time and differences resulting from certain environmental factors.

3. *Correlation of dose and effect*: Teratogens always have a threshold dose, and they can only cause CAs when exceeded in a higher dose.

4. *Site of the teratogenic risk*: The risk of proven and potential human teratogenic drugs is generally low, i.e. under 10%, although a few drugs (thalidomide, folic acid antagonists, anticoagulants) have a higher risk of teratogenicity.

5. *Individual sensitivity*: Not even proven teratogens cause fetal harms in the critical period in every case. The human genome's qualities are the primary underlying factor to individual sensitivity; however, on investigating teratogenicity, both the pregnant woman's and the fetus' genomes influence sensitivity.

Out of the **research methods** concerning the origin of **congenital anomalies**, *animal testing* can only be used as a model, and the results cannot be directly extrapolated to the human species. For example, thalidomide did not prove to be teratogenic in pregnant rats, whereas in humans, when given between post-conceptual days 20 and 35, even a single dose proved to be teratogenic.

Case reports of *clinical observations* are published for rare cases with results that catch attention rather than are suited for generalisation. On processing subject materials, selection bias and the lack of adequate controls are also a problem. Randomised controlled trials (RCT) are made more difficult or prevented altogether by ethical consideration precisely in pregnant women. In *epidemiological research*, descriptive methods examine the incidence and prevalence, as well as demographic features of a given disorder (CA) (e.g. HCAR). Analytical epidemiological studies seek to compare cases and their corresponding controls by comparing the exposure history of the affected and healthy groups. Healthy control is given by most similar individuals, the so-called "*matched controls*". The correspondence between the outcome and the exposure can be statistically tested, and a benefit of this method is that the probability of a given teratogen actually generating the disorder can be defined. A drawback is, however, that the affected persons recall the medical history inaccurately, and that it is difficult to select matched controls.

More useful are the so-called prospective studies that eliminate the drawbacks of recall bias and the lack of adequate matched controls. In such cases, the groups are composed from a prospectively recorded exposures – instead of the outcome. The frequency of CA is compared between groups that were assumed to be exposed and not to be exposed to a teratogen.

## **MATERIALS AND METHODS**

### **1. Hungarian Case-Control Surveillance of Congenital Abnormalities (HCCSCA)**

#### **Subjects of HCCSCA**

Three groups are evaluated in HCCSCA:

1. The first step is to identify the relevant *cases* from the data set of the Hungarian Congenital Abnormality Registry (HCAR). CA cases are reported mainly by obstetricians, pediatricians and pathologists. Cases with CA include live-born infants, stillborn fetuses and electively terminated pregnancy due to malformed fetuses. Cases with CAs were selected for the HCCSCA who were reported after three months of birth or termination of pregnancy (These cases comprised 77% of the HCAR). This short interval between the birth or pregnancy termination and data collection increases the accuracy and effectiveness of the information about the history of pregnancies and can reduce the recall bias (77% of cases were reported during this time window to the HCAR). Three mild CAs were excluded, because their causes were known. In addition CA syndromes with preconception origin were also excluded.
2. The appropriate *controls* to CA cases are selected from the National Birth Registry of the Central Statistical Office. In general, two newborn infants without CA were matched to every case according to sex, birth week, and the district of parents' residence
3. The *abnormal controls* of CA cases were newborns with Down's syndrome. Their syndrome is caused by meiotic non-disjunction of chromosome 21, not by a teratogenic agent. Furthermore, we can reckon with the same recall bias at their mothers, as at mothers of CA cases.

#### **Methods of HCCSCA**

The mothers of cases received an information package by mail. It contained information about the CA, a post-paid structured questionnaire (+ a memory aid: a list of drugs and diseases), and printed informed consent.

Three sources of information were used to obtain exposure data from the mothers of cases and controls in the HCCSCA:

##### *1/Prospective, medically recorded data*

The mothers were asked to send their antenatal maternity logbooks and all other medical records (discharge summaries) regarding their respective pregnancies and the newborns.

##### *2/Retrospective maternal information*

Mothers were asked to complete a structured questionnaire that gave retrospective maternal information about medication during pregnancy. These data included the trade name of the drugs, the dosage and the gestational period during which they were used. In addition, to refresh their memory, mothers were also asked to read an enclosed list of drugs and diseases

before they replied. The questionnaire requested information, among others, on potential confounding factors, as maternal age, birth order, marital and employment status of mothers, smoking and drinking alcohol during pregnancy, pregnancy complications.

### *3/Supplementary information from district nurses*

Regional district nurses were asked to visit and to question all nonrespondent families of cases and 200 nonrespondent control families at home, in addition to obtain their medically recorded data from antenatal maternity logbook, discharge summaries, etc..

The procedure of data collection in the HCCSCA was changed in 1997; therefore in this dissertation I evaluated only the data set of 17 years between 1980 and 1996. I tried to identify possible associations between the low clinical doses of 4 drugs (amobarbital, glutethimide, promethazine and a combination of the four, Tardyl<sup>®</sup>) used during pregnancy and congenital abnormalities.

## **Self-poisoning during pregnancy as a model for teratogenic risk estimation of drugs (SPDP)**

All self-poisoned persons in Budapest and in the surrounding area were admitted to the Department of Toxicological Internal Medicine, Korányi Hospital (hereinafter referred to as 'study hospital') between 1960-1993. Pregnant women who survived suicide attempts committed by taking high doses of drugs may represent a unique model for the study of human teratogenicity, fetotoxicity, and mutagenicity of drugs.

### **Periods of the SPDP Project**

1. *Project Period I.* In 1980, admission records and medical files of all female patients in the study hospital between 1960 and 1979 were reviewed. When the self-poisoned pregnant women were identified, a personal file was developed for each which her personal and medical data, as well as data on her lifestyle and self-poisoning – drugs and doses used for self-poisoning, week of pregnancy, etc. – during the study pregnancy were recorded. These pregnant women were later visited at home for a further examination of their pregnancy outcome. The data regarding the exposed child who was born from the study pregnancy were obtained based on the medical records (discharge summaries of their deliveries, etc.), and these mothers were invited with their children for a medical and psychometric examination at our institute in 1981. Non-cooperating families were visited at home by the medical doctors of the project in 1982 and 1983, and exposed children and their sibs were examined; however, only a certain part of these children had psychometric tests.

2. *Project Period II.* Between 1980 and 1984, a psychologist visited the study hospital every other day and contacted all women the ages of 15-50 years to identify pregnant women on the basis of missed menstrual cycle and case history, through a personal interview. A gynaecologist confirmed that these women were, in fact, pregnant. The data for self-poisoned pregnant women were obtained from the personal files, and the psychologist subsequently visited these mothers at home to further evaluate their pregnancy outcome. She examined exposed children and their sibs externally according to the study protocol, and obtained personal and medical data of these children on the basis of available medical documents

(discharge summary of delivery, medical records of paediatrician, etc.). In addition, cognitive and behavioural development was tested in 1 to 3-year old exposed children and their sibs.

3. *Project Period III*. A pregnancy test (blood HCG) was performed at all reproductive-aged self-poisoned women from 1985 to 1993 at the admission to the study hospital. Each of these pregnant women had a personal file, as previously described. Later on, a study worker visited these women at home to further study their pregnancy outcome, obtained data about exposed children and invited these mothers with their exposed child (and their other children) to the institute for a medical and cognitive-behavioural examination between 8 and 12 postnatal months. All missing exposed children were visited at home by the paediatrician and the psychologist.

## Methods

The *socioeconomic* statuses of self-poisoned pregnant women were analyzed on the basis of their employment, educational and marital statuses, and were classified in three categories: high, medium and low socioeconomic statuses. We evaluated the *smoking* habit during the study pregnancy on the basis of the number of cigarettes smoked daily. Regular and hard *drinkers* were defined as mothers consuming from one drink per day to one drink per week, and more than one drink per day, respectively. An estimation of the *dose of drugs* used for self-poisoning was made using three sources:

1. Information obtained from self-poisoned pregnant women.
2. Drug blood levels measured in the study hospital.
3. Clinical severity of intoxication.

*Pregnancy outcomes* were classified into five groups:

1. Miscarriages
2. Stillbirths
3. Live births
4. Termination of pregnancy
5. Chemical pregnancy (positive pregnancy test without any later clinical symptoms of pregnancy), but only in the project period III.

The main goal of the study was to evaluate the *CAs of the exposed children*. The self-poisoned pregnant women took a high dose of drug(s) once on a specific gestational day, and I had to compare this to the critical period of the CAs. That is why the post-conceptual age is used in the study.

Intrauterine fetal growth retardation appeared to be the most sensitive indicator of a *fetotoxic* effect of drugs. The rate of preterm births (less than 35 completed pregnancy weeks) and low birth weight newborns (less than 2500g) were also evaluated.

Evaluation of a possible *neurotoxic* effect of a drug was based on an evaluation of the cognitive and behavioural status.

Because the results of the Budapest Developmental Test were evaluated during all three project periods, in the dissertation I used only this to evaluate *cognitive development* of the exposed children. Children were classified into four groups, based on their IQs:

AM= above mean (IQ 111-120)

M= mean (IQ 90-110)

UM= under mean (IQ 80-89)

VL= very low (IQ 70-79)

Special attention was paid to the detection of exposed children with *mental retardation* (MR), and when diagnosed, international recommendations (IQ less than 70, etc) were followed. Some exposed children with a suspected new diagnosis of MR had a longer follow-up, and the diagnosis of MR was confirmed or was excluded at the onset of their school age by the official experts.

The behavioural scale of the exposed children was also estimated by a Behavioural Style Questionnaire, in the second and third period of the project. Although a behavioural scale was not measured in the first period of the project, mothers informed us of severe behavioural (criminal) deviations for three exposed children.

It was extremely difficult to find adequate controls to these very specific high risk pregnant women who attempted suicide during pregnancy, and for their exposed children. Finally, we used two control groups:

a) The previous and subsequent unexposed children of self-poisoned pregnant women were used as sib controls. Three matching criteria were used for selection of the sib control: age, similar socio-economic status of mothers and the same sex of the exposed child. If an exposed child had more than one sib, one was selected for this pair analysis based on the same sex and nearest birth year. If the exposed children had no siblings, one previous or subsequent child of a self-poisoned pregnant woman with pregnancy termination or miscarriage after the suicide attempt in the study pregnancy was selected as a control.

b) Another population control group was selected for the group of self-poisoned pregnant women. These pregnant women were selected from the data set of the Hungarian Case-Control Surveillance of Congenital Abnormalities between 1980 and 1996, and provided a reference sample representing 1.8% of all Hungarian births.

Statistical analyses were based on the SAS version 8.02 statistical software packages (SAS Institute, Cary, North Carolina). The quantitative variables of self-poisoned pregnant women, exposed children, and sib controls were evaluated by the Student t-test, whereas categorical variables were evaluated using the chi-square test. The prevalence rates of CAs and MR at birth in the exposed children and in their sib controls were compared, and odds ratios (OR) with a 95% confidence interval (CI) were calculated using an unconditional multiple logistic regression model. Evaluation of exposed child-matched sib control pairs was made using the conditional multiple logistic regression model.

## RESULTS

### *Amobarbital*

#### a) Case-control evaluation of clinical doses

The case group consisted of 22 843 children with CAs and only one pregnant woman was treated by amobarbital. Her child had IUGR and vaginal atresia. In the control group (38 151 children without CA) 2 pregnant women had been given amobarbital treatment. The one case and two controls are not sufficient to evaluate the teratogenic and fetotoxic effect of a clinical dose of amobarbital.

#### b) Case-control evaluation of extremely high doses

Of 1044 pregnant women, 33 used amobarbital for self-poisoning alone or in combination with other drugs. Of these 33 pregnant women, 14 women delivered live-born babies, thus 14 exposed children and the characteristics of their mothers were evaluated.

The pregnant women who attempted suicide with amobarbital had the characteristic traits generally observed for self-poisoned pregnant women, that is, many were young, primiparous, unmarried and of a low socio-economic status. In addition, more than half of them were smokers, and a higher ratio of them were daily or regular drinkers.

The mean birth weight was slightly, but not significantly smaller in exposed children than in their sibs. Mean pregnancy age at delivery was similar in the study groups. There were neither pre-term births, nor low birth weights newborns among exposed children.

The average dose of amobarbital used for self-poisoning by the 14 pregnant women was 19 times larger than the usual clinical dose. 9 pregnant women attempted suicide between the 3<sup>rd</sup> and 12<sup>th</sup> post-conceptional weeks that is the critical period for most major CAs. In spite of this, none of the exposed children had CA.

Out of the 18 sibs, two had CAs: microtia and congenital inguinal hernia.

The cognitive status was measured in the case of 11 exposed children and their 10 sibs. One exposed boy with very low cognitive status (an IQ of about 75) was born to a mother who attempted suicide with 4000 mg amobarbital in the 8<sup>th</sup> post-conceptional weeks; this boy had three sibs with above mean cognitive status (about 115 IQ). Because of this, the estimated mean IQ was somewhat but not significantly lower in exposed children than in their sibs.

Two of eight exposed children and three of five sibs showed a mild behavioural deviation. An exposed adopted boy, born to a mother who was a regular drinker and smoker had a serious social problem because of his very aggressive behaviour.

The results showed no teratogenic, fetotoxic or neurotoxic effect of extremely high doses of amobarbital, although the low number of cases means limitation to evaluation.



## *Glutethimide*

### a) Case-control evaluation of clinical doses

The case group consisted of 22 843 children with CAs and 7 pregnant women was treated by glutethimide (based on the HCCSCA database between 1980 and 1996). Five women were treated in the first trimester and two in the sixth month. The distribution of the seven CAs was: two cases of cleft lip ± cleft palate, two cases of hypospadias, one case of cleft palate, one case of esophageal atresia, and one case of cardiovascular CA. The two cases of cleft lip ± cleft palate (OR and 95% CI: 11.1 2.2-57.3) and the two cases of hypospadias (OR and 95% CI: 5.0 1.0-25.9) seemed to have significant association with glutethimide treatment. Analyzing the cases it turned out that only one mother of the cleft lip ± cleft palate cases was treated in the critical period of this CA, the other one finished treatment in the first month. During the critical period of hypospadias (2<sup>nd</sup> and 3<sup>rd</sup> month), none of the mothers was treated with the drug. In the control group (38 151 children without CA), 5 pregnant women had been given a glutethimide treatment, and four of them in the first trimester. There were no pre-term births or low birth weight among children of treated mothers. Although the number of cases means some limitation of the evaluation, but the results showed no teratogenic, fetotoxic or neurotoxic effect of clinical dose glutethimide.

### b) Case-control evaluation of extremely high doses

Of 1044 pregnant women with self-poisoning, 33 used glutethimide alone or in combination with other drugs for their suicide attempts. Sixteen of these 33 pregnant women delivered live-born babies, thus 16 exposed children and the characteristics of their mothers were evaluated.

The mean maternal age and the mean birth order was somewhat higher in pregnant women who attempted suicide during pregnancy than in the reference group of pregnant Hungarian women. More of them were of a low socio-economic status and unmarried. There was a 2.3- and 19.6-fold increase in the proportion of smokers and regular/hard drinkers among self-poisoned pregnant women, respectively, than in reference mothers of the Hungarian population.

Three of the 16 exposed children had CAs, though six exposed children were born to mothers, who attempted suicide between the 3<sup>rd</sup> and 10<sup>th</sup> post-conceptual weeks. One exposed girl had atrial septal defect, with a critical period between pregnancy weeks 4 and 6. Her mother attempted suicide with 5000 mg glutethimide on the sixth pregnancy week and the association between drug exposure and the CA is likely. The other exposed boy had pectus carinatum, a mild CA, with a critical period during the last months of pregnancy. Her mother attempted suicide with 2500mg glutethimide on the 8<sup>th</sup> pregnancy week, so the association between drug exposure and the CA is unlikely. The third exposed child with multiple CAs was born to a mother affected with panic disorder and she was a hard drinker. His multiple CAs included a mild microcephaly and some minor anomalies that were comparable to the features of FAS; the child also had a lower IQ.

The exposed children had 20 sibs, out of them 3 had CAs (craniosynostosis, cong. inguinal hernia and fetal alcohol syndrome (= FAS)). At evaluation of the pairs of 16 exposed children and matched unexposed sibs, three exposed children and three sibs were affected with CA, the difference was not significant.

The mean birth weight of exposed children and their sibs was comparable, but it was significantly higher in exposed children than in their matched sib pairs. This difference can be explained by the shorter mean pregnancy age at delivery of matched sib pairs.

The distribution of different categories of cognitive status and the estimated mean IQ did not differ from each other in the 14 exposed children and their 14 sibs, although one exposed child with FAS had very low IQ. A behavioural scale was established for 12 exposed children and 10 sibs, but no difference was found in the grades of behavioural deviations.

The very high doses of glutethimide taken by the pregnant women associated with a somewhat higher mean birth weight in their live-born babies explained partly by a somewhat longer pregnancy age at delivery. A longer pregnancy age was observed after a clinical dose treatment, too. These data oppose the glutethimide fetotoxic effects.

The results showed no teratogenic, fetotoxic or neurotoxic effect of extremely high doses of glutethimide, although the low number of cases means limitation to evaluation.

### ***Promethazine***

#### a) Case-control evaluation of clinical doses

The case group consisted of 22 843 children with CAs and 3 648 pregnant women (16 %) had been treated with promethazine (based on the HCCSCA database between 1980 and 1996). In the control group (38 151 children without CA) 6 025 pregnant woman had received a promethazine treatment.

The rate of treatment in the case group was not higher than in the control group. In the different groups of CAs there was no difference in the rate of promethazine treatment.

In months II and/or III of the pregnancy, the promethazine treatment was connected to the somewhat higher rate of cleft lip ± cleft palate (OR with 95% CI: 1.5 1.1-2.0) and poly/syndactyly (OR with 95% CI: 1.3 1.0-1.8). The mothers of children with obstructive CAs of the urinary tract received somewhat less frequent treatments of promethazine (OR with 95% CI: 0.3 0.1-0.8). These differences can be explained by the recall bias on comparisons of mothers with affected children and mothers with healthy babies, because the higher risk of these CAs was not found at the evaluation of only medically recorded promethazine treatment, and/or by the effect of multiple testing.

In conclusion, the use of low clinical dose promethazine did not pose a risk for CAs.

#### b) Case-control evaluation of extremely high doses

Of 1044 pregnant women with self-poisoning, 89 used promethazine at their suicide attempts. Of these 89 pregnant women, 32 women delivered live-born babies.

The mean maternal age was lower than in the representative reference sample; however, mean birth order did not show a difference in self-poisoned pregnant women and the referent Hungarian pregnant women. More women in the self-poisoned group were of low socioeconomic status, and there was a 3.3 and a 11.8-fold increase in the proportion of smokers and regular/hard drinkers among them, respectively.

Of 32 exposed children, nine had CAs and this prevalence seems to be very high; however, it is necessary to evaluate these cases in detail. Of 32 self-poisoned mothers, 11 attempted suicide between their post-conceptual weeks 3 and 10, and three of them had exposed children with CAs. Two boys had undescended testis, but in one case it was familiar. The third exposed boy had a complex cardiovascular CA with five minor anomalies. His mother attempted suicide with 28 tablets of promethazine (700mg) and five tablets of Valeriana Composita (containing 100 mg Phenobarbital and 500 mg valerian) in post-conceptual week 10.

Of the other six CAs, two were isolated CAs: gastroschisis with intestinal atresia and bronchial stenosis. The exposed child with gastroschisis was born to a mother who attempted suicide during the 22nd postconceptional week, however critical period of this CA is between weeks 10 and 14. The critical period of bronchial development is between post-conceptual weeks 6 and 8, and the mother of this exposed child attempted suicide in post-conceptual week 36. In addition, four exposed children were affected with multiple CAs that included one mild CA (undescended testis, congenital inguinal hernia, torticollis, and congenital dysplasia of the hip) and some minor anomalies. Of these four exposed children, two had alcoholic mothers; the third mother was epileptic.

Of 34 sibs, five had CAs. Two sibs were affected with congenital dysplasia of the hip, two other sibs had undescended testis, and the fifth one had talipes equinovarus.

Thus, the sib prevalence at birth of CAs also was high. As a result, the rates of total CAs in sibs and exposed children did not significantly differ.

Cognitive status was measured in 25 exposed children and 22 sibs. The distribution of different categories of cognitive status did not show differences between exposed children and their sibs. This finding was reflected in the estimated mean IQ, which also was similar in exposed children and in their sibs. One mentally retarded boy was found among the exposed children; however, his unexposed brother was also mentally retarded, and their mental retardation was caused by X-linked fragile X.

There was no obvious difference in the number and grade of behavioral deviations between 20 exposed children and their 14 sibs.

The mean birth weight and pregnancy age at delivery of exposed children did not differ from the mean birth weight and pregnancy age at delivery of their sibs.

In conclusion, these results showed no teratogenic, fetotoxic or neurotoxic effect of extremely high doses of promethazine.

### ***Tardyl***<sup>®</sup>

*Tardyl*<sup>®</sup> – a hypnotic drug – is a combination of 125mg amobarbital, 125 mg glutethimide and 7.5 mg promethazine.

#### a) Case-control evaluation of clinical doses

The case group consisted of 22 843 children with CAs and 21 pregnant women were treated by Tardyl (based on the HCCSCA database between 1980 and 1996). In the control group (38 151 children without CA) 57 pregnant woman had had a Tardyl treatment. The mothers of CA cases differed from the mothers of controls in socioeconomic characteristics.

The distribution of CA cases: 4 torticollis, 3 neural-tube defects, 3 poly/syndactyly, while the other CAs were only single cases. The neural-tube defects were not related to Tardyl treatment of the pregnant (OR with 95% CI: 1.7 0.5-5.3). Furthermore one pregnant woman started the treatment in her pregnancy month IV, and one in month VI, while the critical period for this CA is pregnancy month II. The poly/syndactyly cases were not related to the Tardyl treatment of the pregnant women (OR with 95% CI: 1.2 0.43-3.7) and one pregnant woman started taking Tardyl in pregnancy month VI, while the critical period for this CA is during months II-III. The four torticollis cases are interesting. In the database of HCCSCA, 315 out of 22 843 cases had torticollis (1.37%), whereas among the 21 cases of Tardyl treatment during pregnancy, 4 children had torticollis (19.05%). This relationship was confirmed by the case-matched control comparison result (OR with CI: 5.9 1.6-11.4). The critical period for production of torticollis is during the last months of pregnancy, and all the mothers received their Tardyl therapy in the second part of their pregnancy. However, all the four mothers mentioned the Tardyl treatment in the personal interview, and it was not recorded in their antenatal care logbooks. Therefore, the relationship can be explained by the recall bias and/or by the effect of multiple testing.

In conclusion, clinical doses of Tardyl taken during pregnancy did not increase the rate of CAs, although the higher rate of torticollis needs further investigations.

#### b) Case-control evaluation of extremely high doses

Out of 1044 self-poisoned pregnant women, 74 used Tardyl for their suicide attempts alone or in combination with other drugs. Of these 74 women, 27 delivered live-born babies.

The mean age was lower among mothers who attempted suicide, though their mean birth order was not significantly lower. The lower proportion of married women and higher proportion of low socioeconomic status were also characteristic for self-poisoned pregnant women. There was a 2.8- and 18.5-fold higher rate of smokers and regular/hard drinkers among self-poisoned pregnant women, respectively.

Two exposed children had CAs, namely undescended testis and FAS. The mother of the boy affected with the undescended testis attempted suicide using 40 tablets of Tardyl in pregnancy week 20, however, critical period of undescended testis is during the last months of pregnancy. The exposed boy with FAS had a mild microcephaly and three minor anomalies, and his IQ was 68. His hard drinker mother had a panic disorder, and she attempted suicide with 20 tablets of Tardyl and 10 tablets of glutethimide during post-conceptual week 16.

Six pregnant women attempted suicide between post-conceptual weeks 3 and 10, but none of the exposed children have any CAs.

Of 46 sibs, two were affected with CA: oesophageal atresia with tracheal fistula and FAS. Thus, the prevalence at birth of CAs was not higher in exposed children than in their unexposed sibs. Mean birth weight and pregnancy age at delivery was similar in exposed children and their sibs, although both had lower values than the mean birth weight and pregnancy age at birth of the reference Hungarian newborns.

Of 27 exposed children, eight had the diagnosis of mental retardation. Of their eight mothers, six used only Tardyl for their suicide attempt, one was classified as hard (only her child had FAS) and five as regular drinkers. Of these eight children, six had normal karyotypes; the other two children did not have any visible defects but were housed in a special institution, and thus chromosome examination was not allowed. Of the 46 unexposed sibs, none had mental retardation.

IQ could be tested in 22 exposed children and 20 unexposed sibs. The calculated mean IQ was lower in exposed children than in their unexposed sibs. The timing of suicide attempts showed that the high doses of Tardyl were associated with a high risk for mental retardation when exposure occurred between post-conceptual weeks 14 and 20, i.e. in the second trimester of pregnancy.

In addition, I evaluated the cognitive status of the exposed children and their sibs according to the drinking habits of self-poisoned pregnant women. Exposed children and their sibs who were born to mothers without a drinking habit had a higher mean IQ than exposed children and their sibs born to alcohol drinking mothers. However, a lower mean IQ was also found in exposed children compared to their unexposed sibs who were born to the same non-drinker mothers, and this suggests that there may be a neurotoxic effect of Tardyl.

Behavioral scale of 16 exposed children and their 16 sibs supported the possible neurotoxic effect of Tardyl. Of eight mentally retarded exposed children, five had severe, one had moderate and one had a mild behavioural deviation (it was not possible to estimate behavioural status in another mentally retarded boy), though behavioural deviation in general is not characteristic for children with mental retardation. Two other exposed children had a moderate behavioural deviation. On the other hand, of 16 sibs only one had moderate behavioural deviation. Thus, of 27 exposed children, 8 were mentally retarded (29.6%) and 2 other children had moderate behavioural deviation. Of 46 sibs none were mentally retarded and only one had moderate behavioural deviation (2.1%). If we evaluate these 10 exposed children together, the percentage figure is 37.0% in the group of exposed children, while the corresponding figure was 2.1% in the total group of unexposed sibs based on one child with moderate behavioural deviation (OR with 95% CI: 27.6 3.3-232.4).

Finally it was important to make a comparison of exposed children born to mothers who attempted suicide with Tardyl and with the components of Tardyl separately. There was no mentally retarded exposed child in the group exposed to amobarbital or glutethimide; one exposed child in the group of promethazin was mentally retarded but the boy had a genetic X-linked fragile X chromosome. Furthermore the mean IQ was significantly lower in the group of exposed children born to mothers who attempted suicide with Tardyl.

Six exposed children had severe behavioural deviation; five out of them were born to mothers who attempted suicide with Tardyl, and one to a mother who attempted suicide with amobarbital. Four exposed children had moderate behavioural deviation; three of them were born to mothers who attempted suicide with Tardyl and one was born to a mother who attempted suicide with glutethimide.

The drinking and smoking habits during the study pregnancies were not significantly different in pregnant women who attempted suicide with the three components of Tardyl used separately and together in Tardyl. The mean maternal age and birth order did also not differ significantly among pregnant women who used these drugs and Tardyl for their suicide attempt.

Thus the final conclusion is that maternal exposure to high doses of Tardyl, due to the interaction of its component drugs, may induce mental retardation.

## CONCLUSIONS

Many pregnant women who attempt suicide by taking high doses of drugs survive. This population offers a unique model for the estimation of the teratogenic and fetotoxic/neurotoxic effects of drugs in human pregnancies. In my thesis I have evaluated the effects of low clinical doses of 4 different drugs on fetal development. After that, I have evaluated the effects of extremely high doses of the same four drugs on fetal development. If no CA occurs after use of very high doses of a drug during its critical period, this information supports the drug not being a human teratogen or having a fetotoxic effect. The strength of this model is that pregnant women are hospitalized, providing medically recorded exposure data for use in estimating the potential teratogenicity of the drug studied in humans.

### **New results:**

1. The evaluation of low clinical doses of the four drugs used for treatment during pregnancy, i.e. amobarbital, glutethimide, promethazine or Tardyl did not show a potential human teratogen or fetotoxic effect. The relatively few number of cases, recall bias of mothers and different confounders (maternal diseases, other drugs) represent a certain limitation of the evaluation.
2. There was not a higher rate of CAs in exposed children born to mothers who attempted suicide with amobarbital, glutethimid, promethazine or Tardyl compared with the data of their sibs.
3. The evaluation of cognitive and behavioural effect the very high doses of three drugs studied, i.e. amobarbital, glutethimide, promethazine used for suicide attempt did not show a higher risk for mental retardation and behavioral deviation. However, the combination of the three drugs (amobarbital, glutethimide and promethazine) in Tardyl<sup>®</sup> produced a high risk for mental retardation and very low IQ. Although some drugs can cause mental retardation with specified CAs (e.g. valproat), as far as I know, no drug induced mental retardation without structural birth defect has been described until now.
4. The self-poisoning model seems to be a very effective approach to evaluate the potential teratogenicity and fetotoxicity/neuritoxicity of human exposure to drugs.
5. It would be necessary to establish international collaboration for the evaluation of pregnant women with suicide attempt with drugs, because it would provide a larger population for evaluation, so that human risk of drugs could be even better defined.

### ***Limitations of the method***

There are also limitations of the self-poisoning model, e.g. relatively few pregnant women attempt suicide during the critical period for CAs. In addition, most pregnancies were terminated after the self-poisoning in early pregnancy. Some pregnant women used more than one drug for self-poisoning, making it difficult to differentiate the effect of a single drug.

My theses indicate the interaction of different drugs causing mental retardation.

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