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A Population-Based Case-Control Teratologic Study of Oral Dipyrone Treatment during Pregnancy

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Abstract

Objective: To study the possible human teratogenic effect of oral dipyrone, an antipyretic and analgesic drug treatment during pregnancy.

Design and setting: The analysis of cases with different congenital abnormalities and their matched population controls without congenital abnormalities, in addition to a comparison between cases and malformation controls (Down's syndrome) in the population-based, large data set of the Hungarian Case-Control Surveillance of Congenital Abnormalities, 1980–1996.

Study participants: 22 843 neonates or fetuses with congenital abnormalities (cases), 38 151 matched newborns without congenital abnormalities (population controls) and 834 neonates or fetuses with Down's syndrome (malformation controls).

Main outcome measures: 25 congenital abnormality groups.

Results: 1382 (6%) cases, 1911 (5%) population controls and 74 (8.9%) malformation controls were born to mothers treated with dipyrone during pregnancy. The case-matched population control analysis showed a higher rate of diaphragmatic defect (adjusted prevalence odds ratio [POR] 2.7; 95% CI 1.0, 6.8), cardiovascular malformations (POR 1.3; 95% CI 1.0, 1.7) and other isolated congenital abnormalities (POR 1.8; 95% CI 1.1, 2.9) after oral dipyrone treatment during the second and third months of gestation, i.e. in the critical period for most major congenital abnormalities. However, the evaluation of only medically recorded dipyrone use did not confirm these possible associations. The comparison of dipyrone treatment between 25 congenital abnormalities groups and malformation controls as the referent group also did not show any difference in the dipyrone use during the second and third months of gestation.

Conclusions: The higher occurrence of dipyrone treatment in the case mothers compared with population control mothers can be explained by recall bias and/or chance. However, the higher rate of diaphragmatic congenital abnormalities can be considered as a signal and merits further investigation.

Background

Different nations have different cultures, and before the globalisation of our world different nations had different practices of drug treatments as well. The 'pharmaceutical culture' was determined mainly by the available medicinal products due to – at least in the past – the profile of national pharmaceutical factories, policy of regulatory agencies and the education of medical doctors. A good example of the variation in pharmaceutical culture is the extremely high use of dipyrone (its synonymic names include metamizole, methamizole sodium, noramidazophene and novamidazophene) in Hungary.[1]

The chemical name of dipyrone is phenyl-dimethyl-pyrazolone-4-methylaminomethane sulfonacid-sodium and this pyrazolone NSAID is used as an analgesic and antipyretic agent. Amidopyrine (amidazophene) and later dipyrone were marketed in the first decades of the 20th century.[2] However, after the detection of agranulocytosis due to amidopyrine and dipyrone in the 1930s, both drugs were withdrawn from the drug market in several countries including the US and UK.[3-6] However, dipyrone was used further in some countries of Europe, South America and the Middle East. Agranulocytosis was never observed after the use of these pirazolone derivatives in Hungary.[7]

Later, Japanese studies indicated that dipyrone had a carcinogenic effect in mice,[8] in addition to its mutagenic effect in *Salmonella typhimurium* and tumour-promoting potential in male F344 rats.[9] Recently, an increased risk for Wilm's tumour[10] and infantile acute leukaemia[11] have been reported in the offspring of women taking dipyrone during pregnancy.

Dipyrone is rapidly converted by hydrolysis to pharmacologically active metabolites in the gastrointestinal tract after oral administration.[12,13] Dipyrone crosses the placental barrier[12] and can block cell division in the placenta, mainly in the phase of formation and placental differentiation.[13] Catalan et al.[14] reported an association between dipyrone use during pregnancy and both oligohydramnion and premature closure of ductus arteriosus (Botalli), and these associations were explained by the inhibitor effect of dipyrone for prostaglandin synthetase.

The antipyretic, analgesic and anti-inflammatory effect of dipyrone has been used frequently alone or in combination with other drugs for the treatment of different diseases or symptoms in Hungary since 1922. In 2005, we decided to evaluate the teratogenic risk of dipyrone in the large population-based data set of the Hungarian Case-Control Surveillance of Congenital Abnormalities (HCCSCA),[15] because the results of controlled epidemiological studies had not been published. However, Bar-Oz et al.[16] reported a case-control study based on 108 pairs in 2005 and their conclusion was that exposure to dipyrone during the first trimester of pregnancy is probably not associated with a significantly increased risk for congenital abnormalities. However, their conclusion was based on three and two cases with major congenital abnormality in the exposed and unexposed groups, respectively.

Methods

Cases with congenital abnormalities were selected from the Hungarian Congenital Abnormality Registry (HCAR) for inclusion in the HCCSCA study.[17] Notification of congenital abnormalities is mandatory for physicians in Hungary. Most cases with congenital abnormality are reported by obstetricians (practically all deliveries in Hungary occur in inpatient obstetric clinics and birth attendants are obstetricians) and paediatricians (who are working in the neonatal units of inpatient obstetric clinics, in addition to various in- and outpatient paediatric clinics) to the HCAR. Autopsy during the study period was obligatory for all infant deaths and usual for stillborn fetuses. Pathologists sent a copy of the autopsy report to the HCAR if defects were identified in stillborn fetuses and infant deaths. Fetal defects diagnosed in the antenatal diagnostic centres with or without termination of pregnancy have also been included in the HCAR since 1984. Cases with isolated minor anomalies were recorded but not evaluated in the HCAR. The recorded total (birth and fetal) prevalence of cases with congenital abnor-

malities diagnosed from the second trimester of pregnancy through to first postnatal year was 35 per 1000 informative offspring (liveborn infants, stillborn fetuses and electively terminated malformed fetuses after prenatal diagnosis) during the 17-year study period and approximately 90% of major congenital abnormalities were reported to the HCAR.[18]

HCCSCA Procedure

Case and Control Identification

The first step of the HCCSCA procedure included the identification of cases and controls.

Case Identification

Cases with congenital abnormalities that were reported during the first 3 months after birth or pregnancy termination were identified from the HCAR data set. These cases comprised 77% of the HCAR.[17] Three degrees of severity were differentiated among congenital abnormalities: lethal (e.g. anencephaly), severe (e.g. cleft lip with cleft palate), and major or mild (e.g. torticollis). However, three mild congenital abnormalities (congenital dysplasia of the hip based on the Ortolani click, congenital inguinal hernia and large haemangiomas) and congenital abnormality syndromes of pre-conceptional Mendelian or chromosomal origin were excluded. The exception was Down's syndrome.

Population Controls

Population controls were defined as newborn infants without congenital abnormalities and were selected from the Hungarian Birth Registry of the Central Statistical Office. In general, two newborn infants without congenital abnormalities were matched with every case according to sex, birth week and district of parents' residence. However, three population controls were selected for each case between 1986 and 1992.

Malformation Controls

The so-called malformation controls were cases affected with Down's syndrome and they were also selected from the data set of the HCAR reported during the first 3 months after birth or pregnancy termination for the HCCSCA to estimate the effect of recall bias.

Data Collection

The second step of the HCCSCA was to obtain necessary data from three sources.

Retrospective Maternal Self-Reported Information

A post-paid questionnaire and explanatory letter, together with a list of medicines (drugs and pregnancy supplements) and diseases, were mailed to parents immediately after the selection of cases and controls. The questionnaire requested information on pregnancy complications, maternal diseases and medicine intakes during pregnancy according to gestational month. To standardise the answers, mothers were asked to read the enclosed lists of medicines and diseases as a memory aid before they replied. In addition, a printed informed consent form regarding permission to record the name and address of cases in the HCCSCA was sent to mothers of cases.

Prospective Medically Recorded Data

Mothers were asked to send their antenatal care logbook, other medical records concerning their diseases during pregnancy and details of their child's congenital abnormality. Antenatal care is mandatory for pregnant women in Hungary (if a woman does not visit antenatal care, she does not receive the maternity grant and leave); thus, nearly 100% of pregnant women visited antenatal care during the study period with a first visit within 6–12 weeks of gestation and with an average of seven visits during their pregnancy.

The time period between the end of pregnancy and return of the information package (questionnaire, logbook, consent form) was 3.5 ± 1.2, 5.2 ± 2.9 and 3.8 ± 2.0 months for cases, population controls and malformation controls, respectively. Among respondents, antenatal care logbooks were available in 88.4% of cases, in 93.8% of population controls and in 90.8% of malformation controls. Antenatal care books that were not submitted may have been lost by the mother or requested by the cases' doctor (particularly if the child was affected with a disease). Informed consent forms were signed and returned by 98% of case mothers. Names and addresses were not recorded for controls in the HCCSCA study.

Complementary Data Collection

Regional nurses were asked to visit and question all non-respondent case and malformation control mothers but only 200 non-respondent population control mothers. The same questionnaire was used through a personal interview completed by the evaluation of available medical records. Unfortunately, the ethics committee considered that this follow-up would be disturbing to the parents of all healthy population control children.[19]

Dipyrone Analysis

In this study, the data set of the HCCSCA was evaluated for the years 1980–1996 because the method of data collection was changed after 1996 and the recent data set has not been validated.

The flow of cases from the HCAR and population controls from the Hungarian Birth Registry of the Central Statistical Office to the HCCSCA was shown previously.[20] Thus, the total number of cases was 23 721, whereas the total number of matched population controls was 45 946. A total of 22 843 (96.3%) cases with congenital abnormalities were evaluated in the data set and the necessary information was available due to the response in 20 021 (84.4%) cases and home visits in 2822 (11.9%) cases. The data set of population controls contained 38 151 newborns (83.0%): 37 951 (82.6%) had response and 200 (0.4%) were visited at home. The group of malformation controls included 834 (96%) fetuses or newborn infants with Down's syndrome due to response in 751 (86%) and home visit in 83 (10%) malformation cases.

Evaluation of Dipyrone Exposure

Dipyrone (Algopyrin® [1], Chinoin, Novalgin®, Aventis Pharma) was used for oral treatment as a tablet containing 500mg or as parenteral treatment in an ampoule containing 1000mg or 2500mg of dipyrone. According to the data set of the HCCSCA, pregnant women were not treated parenterally by dipyrone. The recommended daily dose for oral dipyrone treatment is 1–2 tablets three times daily, i.e. 1.5–3 g/day, because dipyrone has a short half-life.[1]

The following three groups of exposures were differentiated:

- Only data from the antenatal care logbook (antenatal care obstetricians are obliged to record all prescribed drugs for pregnancy-related complications and diseases in the logbook) and/or other medical records, mainly the discharge summary.
- Only data from the questionnaire (including drugs used for treatment of diseases unrelated to pregnancy prescribed by general practitioners or other physicians and medicines including over-the-counter products taken according to the woman's personal choice).
- Concordant data from both the medical records and questionnaire.

Two groups were differentiated in the analysis: dipyrone alone and dipyrone plus other medicines. Combination products containing dipyrone were not evaluated in the study.

The gestational time was calculated from the first day of the last menstrual period and the following three time periods were considered.

- The first month of pregnancy comprising the first 2 weeks before conception and the next 2 weeks comprising the preimplantation and implantation periods of zygotes/blastocysts including stem cells. Thus, congenital abnormalities cannot be induced by short-term environmental agents during the first month of gestation, explaining the 'all-or-nothing effect' rule.[21]
- The second and third months of gestation cover organogenesis, which is the most sensitive, so-called 'critical period', for most major congenital abnormalities. All pregnant women who had dipyrone exposure during the second and/or third months, irrespective of when they started taking dipyrone were included in this period.
- The fourth to ninth months of gestation.

Among potential confounding factors related to the mother, age, birth order (number of live and still births), marital and employment status (as indicators

1 The use of trade names is for product identification purposes only and does not imply endorsement.

of socio-economic status), acute and chronic disorders and use of other medicinal products were considered.

Statistical Analysis

Statistical analyses were performed using the software package SAS version 8.02 (SAS Institute Inc., Cary, NC, USA). Three sources of exposure information were evaluated separately; first we report the total (both medically recorded and maternal information) occurrence of dipyrone treatment, but finally only medically documented data were considered at the evaluation of our results. The occurrence of dipyrone treatment was compared in the three study groups and crude prevalence odds ratios (POR) with 95% CI were calculated.

Potential confounders, such as maternal age, birth order, maternal marital and employment status were evaluated using the Student's t-test for quantitative variables and the Chi-squared (χ^2) test for categorical variables. Prevalence of pregnancy complications and maternal diseases was also compared using unadjusted POR with 95% CI in the study groups.

Use of other drugs and pregnancy supplements during the entire pregnancy were compared among the three study groups treated with dipyrone in logistic regression models. The prevalence of dipyrone treatment during the entire pregnancy and in the second and/or third months of gestation in different congenital abnormality groups was compared with the frequency of dipyrone treatment in their 1–3 matched population controls, and adjusted POR with 95% CI were evaluated in conditional logistic regression models.

To reduce the recall bias, the prevalence of medically recorded dipyrone treatment in different congenital abnormality groups was compared with the frequency of medically recorded dipyrone treatment in the matched population control group. Finally, we compared the prevalence of dipyrone treatment in the malformation control group as referent with the prevalence of dipyrone treatment in the specific congenital abnormality groups in unconditional logistic regression models.

Results

The number of cases with different congenital abnormalities was 22 843 and oral dipyrone treatment was recorded in 1382 (6.0%) case mothers. During the study period, the number of total births in Hungary was 2 146 574; thus, 38 151 population controls represented 1.8% of the Hungarian births. The number of population controls who had mothers receiving oral dipyrone treatment during pregnancy was 1911 (5.0%); adjusted POR 1.2; 95% CI 1.1, 1.3. Of 834 malformation controls, 74 (8.9%) had mothers who received oral dipyrone during pregnancy (adjusted POR 0.6; 95% CI 0.5, 0.8). Parenteral treatment of dipyrone was not recorded in the data set of the HCCSCA.

In general, 1.5 g/day of oral dipyrone dose was used with a range of 0.5–4.0mg. The mean duration of treatment was 2.5 weeks for cases, 2.8 weeks for population controls and 3.1 weeks for malformation controls; however, a limited number of pregnant women had only 1 day of treatment.

Use of dipyrone alone was rare (n = 46, 3.3% for cases; n = 65, 3.4% for population controls and n = 3, 4.1% for malformation controls). These differences were not significant, and dipyrone treatment alone or with other medicines was combined in further analysis.

Medically-recorded treatment with dipyrone was available for 261 of 1382 (18.9%) case mothers, for 388 of 1911 (20.3%) population control mothers (χ^2 = 1.95; p = 0.16) and for 18 of 74 (24.3%) malformation control mothers (χ^2 = 1.08; p = 0.30).

Potential confounders are presented in table I. Mean maternal age was somewhat lower in the dipyrone-treated case mothers due to the higher proportion of younger women (≤24 years) compared with treated population control mothers. On the other hand, mean birth order was higher in the group of case mothers. Malformation controls showed the characteristics of Down's syndrome mothers, i.e. higher mean maternal age and birth order.

There were some differences in the employment status of mothers: the proportion of professional and managerial workers was smaller, whereas the proportion of semi-skilled workers and housewives was

Table I. Basic characteristics of mothers [n (%)] treated or untreated with dipyrone in the three study groups

Potential maternal confounders	Cases		Population controls		Malformation controls	
	untreated (n = 21 461)	treated (n = 1382)	untreated (n = 36 240)	treated (n = 1911)	untreated (n = 760)	treated (n = 74)
Maternal age (yr)[a]						
≤24	10 254 (47.8)	691 (50.0)	17 162 (47.4)	832 (43.5)	234 (30.8)	26 (35.1)
25–29	6 726 (31.3)	428 (31.0)	12 181 (33.6)	704 (36.8)	195 (25.7)	19 (25.7)
≥30	4 481 (20.9)	263 (19.0)	6 897 (19.0)	375 (19.6)	331 (43.6)	29 (39.2)
mean ± SD[b]	25.5 ± 5.3	25.3 ± 5.1	25.4 ± 4.9	25.7 ± 4.8	29.1 ± 7.4	28.3 ± 7.3
Birth order[c]						
1	10 051 (46.8)	657 (47.5)	17 349 (47.9)	860 (45.0)	271 (35.7)	24 (32.4)
≥2	11 410 (53.2)	725 (52.5)	18 891 (52.1)	1 051 (55.0)	489 (64.3)	50 (67.6)
mean ± SD[d]	1.86 ± 1.14	1.84 ± 1.14	1.74 ± 0.94	1.76 ± 0.87	2.23 ± 1.44	2.51 ± 2.20
Unmarried[e]	1 212 (5.6)	57 (4.1)	1 414 (3.9)	57 (3.0)	44 (5.8)	5 (6.8)
Employment status[f]						
professional	1 759 (8.2)	142 (10.3)	4 093 (11.3)	260 (13.6)	72 (9.5)	7 (9.5)
managerial	4 646 (21.6)	322 (23.3)	9 597 (26.5)	537 (28.1)	182 (23.9)	18 (24.3)
skilled worker	5 925 (27.6)	404 (29.2)	11 102 (30.6)	588 (30.8)	178 (23.4)	22 (29.7)
semi-skilled worker	3 632 (16.9)	237 (17.1)	5 533 (15.3)	250 (13.1)	144 (19.0)	9 (12.2)
unskilled worker	1 432 (6.7)	71 (5.1)	1 779 (4.9)	80 (4.2)	48 (6.3)	6 (8.1)
housewife	2 020 (9.4)	108 (7.8)	1 977 (5.5)	61 (3.2)	61 (8.0)	6 (8.1)
others	2 047 (9.5)	98 (7.1)	2 159 (6.0)	135 (7.1)	75 (9.9)	6 (8.1)

a Comparison between treated groups: $\chi^2 = 15.4$, $p = 0.0004$ for population controls; $\chi^2 = 18.0$, $p = 0.0001$ for malformation controls.

b Comparison between treated groups: $t = 2.6$, $p = 0.009$ for population controls; $t = 3.6$; $p = 0.0007$ for malformation controls.

c Comparison between treated groups: $\chi^2 = 2.1$, $p = 0.15$ for population controls; $\chi^2 = 6.4$, $p = 0.01$ for malformation controls.

d Comparison between treated groups: $t = 2.1$, $p = 0.03$ for population controls; $t = 2.6$, $p = 0.01$ for malformation controls.

e Comparison between treated groups: $\chi^2 = 3.1$, $p = 0.08$ for population controls; $\chi^2 = 1.2$, $p = 0.27$ for malformation controls.

f Comparison between treated groups: $\chi^2 = 58.9$, $p < 0.0001$ for population controls; $\chi^2 = 2.4$, $p = 0.88$ for malformation controls.

SD = standard deviation.

larger in the treated case group taking dipyrone than in the treated population control mothers. (Generally, in Hungary, housewives have a lower socioeconomic status.) However, there was no significant difference in the distribution of maternal employment status between treated case and malformation control mothers.

The prevalence of pregnancy complications is shown in the study groups in table II. Polyhydramnios showed a higher occurrence in the treated case than in the treated population control groups. On the other hand, the occurrence of threatened abortion and pre-eclampsia was lower in the treated case group than in the treated population control group. There was no difference in these variables between treated case and malformation control groups.

Significant differences were not found in the prevalence of all but one acute or chronic maternal diseases among the three treated study groups. The exception was the group with influenza and the common cold (in general, with secondary complications), which showed a higher occurrence in the treated case group.

Among the other frequently used drugs, only two: aspirin (acetylsalicylic acid) [n = 201, 14.5% vs n = 204, 10.7%; POR 1.4; 95% CI 1.2, 1.8], and aminophenazone plus carbromal (n = 158, 11.4% vs n = 125, 6.5%; POR 1.8; 95% CI 1.4, 2.4) were used more frequently by case mothers compared with population control mothers treated with dipyrone. However, there was no difference in the use of any drugs between case and malformation control mothers.

Among pregnancy supplements, iron-containing tablets and folic acid were used somewhat less frequently by the treated case (n = 822, 59.5% and n = 641, 46.4%) than by the treated population control (n = 1255, 65.7% and n = 1024; 53.6%) mothers (POR 0.8; 95% CI 0.7, 0.9 and POR 0.7; 95% CI 0.6, 0.9), respectively. However, there was no difference in the use of pregnancy supplements between case and malformation control mothers.

The prevalence of maternal oral dipyrone treatment in cases with different congenital abnormality groups was compared with the use of this drug

Table II. Prevalence of pregnancy complications [n (%)] in the study groups

Pregnancy complications	Cases		Population controls			Malformation controls		
	untreated (n = 21 461)	treated (n = 1 382)	untreated (n = 36 240)	treated (n = 1 911)	comparison [POR (95% CI)]	untreated (n = 760)	treated (n = 74)	comparison [POR (95% CI)]
Threatened abortion	3 261 (15.2)	240 (17.4)	6 126 (16.9)	386 (20.2)	0.8 (0.7, 0.9)	121 (15.9)	9 (12.2)	1.5 (0.7, 3.1)
Nausea, vomiting (excessive)	1 583 (7.4)	163 (11.8)	3 622 (10.0)	247 (12.9)	0.9 (0.7, 1.1)	57 (7.5)	4 (5.4)	2.3 (0.8, 6.5)
Pre-eclampsia, eclampsia[a]	1 682 (7.8)	100 (7.2)	3 037 (8.4)	184 (9.6)	0.7 (0.6, 0.9)	55 (7.2)	4 (5.4)	1.4 (0.5, 3.8)
Threatened preterm delivery[b]	2 454 (11.4)	180 (13.0)	5 182 (14.3)	278 (14.5)	0.9 (0.7, 1.1)	95 (12.5)	10 (13.5)	1.0 (0.5, 1.9)
Placental disorders[c]	266 (1.2)	28 (2.0)	540 (1.5)	52 (2.7)	0.7 (0.5, 1.2)	9 (1.2)	2 (2.7)	0.7 (0.2, 3.2)
Oligohydramnios	30 (0.1)	3 (0.2)	11 (0.0)	3 (0.2)	1.4 (0.3, 6.9)	1 (0.1)	0 (0.0)	
Polyhydramnios	180 (0.8)	32 (2.3)	170 (0.5)	21 (1.1)	2.1 (1.2, 3.7)	13 (1.7)	2 (2.7)	0.9 (0.2, 3.6)
Gestational diabetes mellitus	131 (0.6)	10 (0.7)	253 (0.7)	17 (0.9)	0.8 (0.4, 1.8)	6 (0.8)	1 (1.4)	0.5 (0.1, 4.2)
Anaemia	3 020 (14.1)	220 (15.9)	6 054 (16.7)	302 (15.8)	1.0 (0.8, 1.2)	86 (11.3)	12 (16.2)	1.0 (0.5, 1.8)

a Including pregnancy hypertension, oedema and albuminuria.

b Including cervical incompetence.

c Including placenta previa, premature separation of placenta, antepartum haemorrhage.

POR = prevalence odds ratios.

Table III. Two different analyses, using the matched population controls and unmatched malformation controls as reference, to estimate the association between dipyrone treatment during the second and/or third months of pregnancy and different congenital abnormalities (CAs)

Study groups	Total [n]	Dipyrone treatment [n (%)]	Comparison with matched population controls [adjusted POR[a] (95% CI)]	Comparison with malformation controls [adjusted POR[b] (95% CI)]
Isolated CAs				
neural-tube defects	1 202	32 (2.7)	1.5 (0.9, 2.6)	0.8 (0.4, 1.3)
cleft lip ± palate	1 374	40 (2.9)	1.2 (0.8, 2.0)	0.8 (0.5, 1.4)
posterior cleft palate	582	21 (3.6)	1.9 (0.9, 3.6)	1.1 (0.6, 2.0)
esophageal atresia/stenosis	217	2 (0.9)	1.7 (0.2, 12.8)	0.3 (0.1, 1.2)
congenital pyloric stenosis	241	5 (2.1)	0.7 (0.2, 2.0)	0.7 (0.2, 1.8)
intestinal atresia/stenosis	153	3 (2.0)	1.1 (0.2, 5.9)	0.6 (0.2, 2.0)
rectal/anal atresia/stenosis	220	7 (3.2)	1.6 (0.5, 5.0)	1.1 (0.5, 2.7)
renal a/dysgenesis	104	1 (1.0)	0.5 (0.1, 4.9)	0.3 (0.0, 2.4)
obstructive urinary CAs	271	5 (1.8)	0.9 (0.3, 3.3)	0.6 (0.2, 1.6)
hypospadias	3 038	73 (2.4)	1.2 (0.8, 1.6)	0.8 (0.5, 1.3)
undescended testis	2 051	43 (2.1)	1.2 (0.8, 1.8)	0.7 (0.4, 1.2)
exomphalos/gastroschisis	238	6 (2.5)	1.3 (0.4, 3.7)	0.8 (0.3, 2.0)
microcephaly, primary	109	2 (1.8)	0.8 (0.1, 4.6)	0.6 (0.1, 2.7)
congenital hydrocephaly	314	7 (2.2)	1.4 (0.5, 3.9)	0.7 (0.3, 1.6)
eye CAs	99	4 (4.0)	1.2 (0.3, 5.3)	1.2 (0.4, 3.6)
ear CAs	354	8 (2.3)	1.6 (0.5, 5.1)	0.8 (0.3, 1.7)
cardiovascular CAs	4 479	128 (2.9)	1.3 (1.0, 1.7)	0.9 (0.6, 1.4)
CAs of genital organs	123	5 (4.1)	2.0 (0.5, 8.3)	1.4 (0.5, 3.8)
clubfoot	2 424	47 (1.9)	1.1 (0.8, 1.6)	0.7 (0.4, 1.1)
limb deficiencies	548	20 (3.6)	1.7 (0.9, 3.2)	1.1 (0.6, 2.1)
poly/syndactyly	1 744	36 (2.1)	1.1 (0.7, 1.7)	0.7 (0.4, 1.2)
CAs of skeletal system	211	4 (1.9)	2.4 (0.6, 10.4)	0.7 (0.2, 2.0)
diaphragmatic CAs	243	11 (4.5)	2.7 (1.0, 6.8)	1.3 (0.6, 2.8)
other isolated CAs	1 155	38 (3.3)	1.8 (1.1, 2.9)	1.1 (0.6, 1.8)
Multiple CAs	1 349	45 (3.3)	1.4 (0.9, 2.2)	1.0 (0.6, 1.7)
Total cases	22 843	593 (2.6)	1.3 (1.2, 1.5)	0.8 (0.6, 1.2)
Total population controls	38 151	736 (1.9)		
Malformation controls	834	26 (3.1)		

a Matched POR adjusted for maternal employment status and influenza-common cold during the second and/or third month of pregnancy in conditional logistic regression model.

b Unmatched POR adjusted for maternal age and employment status, birth order and influenza-common cold during the second and/or third month of pregnancy in unconditional logistic regression model.

POR = prevalence odds ratios.

during the second to third gestational months (as the critical period for most major congenital abnormalities) in their all matched population controls (table III). Among the 25 congenital abnormality groups studied, three congenital abnormality groups: cardiovascular, diaphragmatic and other isolated congenital abnormalities showed a higher use of dipyrone.

Cardiovascular congenital abnormalities comprise several congenital abnormalities with different clinical manifestations and origin, and these subgroups are shown in table IV. There is no difference between expected number of different cardiovascular congenital abnormality entities based on the data set of the HCCSCA and observed number.

There are five types of diaphragmatic congenital abnormalities: (i) Bochdalek (posterolateral defect

Table IV. The expected and observed number of different cardiovascular congenital abnormalities (CAs) after the treatment of dipyrone during the entire pregnancy (T) or in the second and third month of gestation (II-III) and their comparison using χ^2_1 test

Groups of cardiovascular CAs	Expected			Observed		p-Value	
	%	n^T	n^{II-III}	n^T	n^{II-III}	n^T	n^{II-III}
Common truncus	0.7	2	1	1	0	0.56	0.32
Transposition of great vessels	3.4	9	4	10	6	0.81	0.52
Tetralogy of Fallot	3.9	11	5	8	4	0.48	0.73
Ventricular septal defect	34.9	95	45	111	47	0.16	0.79
Atrial septal defect, type II	10.4	28	13	25	13	0.66	1.00
Hypoplastic left heart	2.6	7	3	4	2	0.36	0.65
Patent ductus arteriosus	3.9	11	5	11	6	1.00	0.76
Coarctation of aorta	2.7	7	3	3	1	0.20	0.31
Other CAs of aorta and aortic valves	2.0	6	3	10	4	0.31	0.70
CAs of pulmonary artery and valves	5.9	16	8	15	7	0.85	0.79
Other isolated cardiovascular CAs	3.9	11	5	9	3	0.65	0.47
Complex cardiovascular CAs	7.1	19	9	18	9	0.86	1.00
Unspecified cardiovascular CAs	18.6	51	24	53	26	0.83	0.75
Total	100.0	273	128	273	128		

in the diaphragm); (ii) Morgani (anterolateral defect of diaphragm); (iii) total relaxation of diaphragm with the eventration of abdominal organs; (iv) hiatus hernia causing gastro-oesophageal reflux; and (v) pericardial diaphragmatic defect. The percentage distribution of these types of diaphragmatic congenital abnormality was 85%, 3%, 7%, 4% and 1%, respectively.

The distribution of two or more congenital abnormalities within the group of 38 other isolated congenital abnormalities who had mothers with dipyrone treatment during the second to third months of pregnancy was the following: torticollis 8; teratoma 3; malrotation of colon 3; lung hypo/dysplasia 2 and atresia of bile duct 2. All other isolated congenital abnormalities occurred only in one case.

In the next step, only medically recorded dipyrone treatments were evaluated during the second to third months of pregnancy. There was no congenital abnormality group with higher dipyrone treatment during this critical period of most major congenital abnormalities in case mothers than in population control mothers including cardiovascular congenital abnormalities (POR 1.0; 95% CI 0.5, 1.9), diaphragmatic congenital abnormalities (POR 1.8; 95% CI 0.3, 11.4) and other isolated congenital abnormalities (adjusted POR 2.0; 95% CI 0.6, 7.1).

The use of dipyrone during pregnancy was compared between the case mothers with different congenital abnormalities and malformation control mothers (table III). There was no congenital abnormality group with higher use of dipyrone during the second and third months of gestation in any congenital abnormality group.

We evaluated birth weight and gestational age in control newborns (congenital abnormalities may have a more drastic effect on these birth outcomes than dipyrone itself). There was no difference in the mean birth weight and gestational age of 1911 and 36 240 newborn infants born to mothers with or without dipyrone treatments (3288 ± 504g vs 3275 ± 511g; 39.4 ± 2.0 weeks vs 39.4 ± 2.0 weeks). The rate of preterm births (8.5% vs 9.2%) and low birth weight (5.8% vs 5.7%) was also similar between the two subgroups.

Discussion

We estimated the risk of congenital abnormality associated with oral dipyrone treatment during the second and/or third month of pregnancy by comparing cases with congenital abnormalities with matched population and malformation controls. Treatment with oral dipyrone at the usual therapeu-

tic dose during pregnancy presents very little, if any, teratogenic risk for the embryo.

The strengths of the HCCSCA are: (i) the large, population-based data set, including 3367 pregnant women who received oral dipyrone treatment; (ii) the matching of cases with congenital abnormalities and their controls without congenital abnormalities; (iii) malformation controls to estimate recall bias; (iv) the prospective and medically recorded data of dipyrone treatment in 667 pregnant women; (v) the knowledge of potential confounders; (vi) exposure time data, with which we were able to evaluate the critical period of most congenital abnormalities, i.e. the second and third month of gestation, instead of the old fashioned first trimester concept; and (vii) the good validity of congenital abnormality diagnoses reported by medical doctors and checked in the HCAR.

In addition, the recent medical examinations helped us to exclude misdiagnosed congenital abnormalities and to improve the quality of congenital abnormality-diagnosis, e.g. in the subgroups of cardiovascular congenital abnormalities. The HCCSCA is the largest case-control data set of its type in the world and population-based sampling makes risk assessment possible.

However, this data set also has weaknesses. Dipyrone use was only based on retrospective maternal information for 81% of cases, 80% of population controls and 76% of malformation controls. The primary reason of this pattern is that dipyrone in tablet form was prescribed by general practitioners and, in general, the antenatal care logbook included only drugs prescribed by obstetricians. Thus, we have to consider recall bias in the population control group, which may cause spurious associations with biased POR up to a factor of 1.9.[22] Since the birth of an infant with a congenital abnormality is a serious traumatic event for most mothers, they try to find a causal explanation such as medicine use during pregnancy.

However, it is possible to exclude recall bias by different approaches. First, dipyrone treatment was evaluated only during the critical period for most major congenital abnormalities, i.e. in the second and third months of gestation because we expect an under-reporting of exposure in both the critical and non-critical periods of congenital abnormalities in the population control group. Second, another more valid source of exposure data was used. The medically recorded data may serve as a reference standard, and when we compared only the medically recorded dipyrone use between the case and population control groups, we were not able to confirm the possible teratogenic effect of dipyrone. Third, we used malformation controls because their mothers had a similar traumatic event to the mothers of cases. This approach helped us to estimate the recall bias of population control mothers.[23] The comparison of case and malformation control groups did not show any teratogenic effect of dipyrone.

Although the response rate was nearly similar in the control and case groups, there was an active follow-up for all nonrespondent case and malformation control mothers, but only 200 nonrespondent population control mothers.

The period between the end of pregnancy and return of the questionnaire was different between the case and population groups ($t = 84.4$; $p < 0.001$). These methodological differences caused some asymmetry in the data sets of cases and population controls, i.e. selection bias. The use of dipyrone in 200 nonrespondent population control mothers visited at home did not differ significantly from the rate of population control pregnant women who did respond.[19]

Another bias may be connected with multiple comparisons, which produces noncausal associations because a significant difference is expected in every 20th estimation as a result of chance.[24] We cannot exclude this chance effect in the previously mentioned three congenital abnormality groups listed in table III.

We know of only one experimental investigation in which the teratogenic effect of dipyrone was checked. Ungthavorn et al.[25] injected this antipyretic drug into pregnant mice in doses up to 1000 mg/kg on days 8, 9 or 10 of pregnancy. Of 68 fetuses receiving 750 mg/kg on day 9, six had exencephaly or encephalocele. Their incidence was not dose de-

pendent and these findings were not confirmed by our study because the occurrence of neural-tube defects was not higher in the informative offspring of case mothers. Our study did also not confirm the previously found association between dipyrone use and oligohydramnios.[14] In fact, we found a higher prevalence of polyhydramnios in case mothers. Cases with Wilm's tumour[10] are not recorded in the HCCSCA because Mendelian entities are excluded.

A higher risk for cardiovascular congenital abnormalities, diaphragmatic congenital abnormalities and other isolated congenital abnormalities was found after oral dipyrone treatment during the second and third month of gestation at the comparison of case and population control groups. However, the risk for cardiovascular congenital abnormalities was only 1.3 (95% CI 1.0, 1.7) but this association was not confirmed at the evaluation of medically documented dipyrone treatment and at the comparison of cases and malformed controls. In addition, we were not able to show any increase in the specific types/groups of cardiovascular congenital abnormalities including patent ductus arteriosus, which showed a higher occurrence in a previous study.[14] Thus, this finding can be explained by chance.

The group of other isolated congenital abnormalities included very heterogeneous congenital abnormalities with different aetiology; therefore, it is difficult to suppose a causal association. Diaphragmatic congenital abnormalities had the highest risk (2.7, 95% CI 1.0, 6.8) based on 11 cases and the limited number of cases explains (i) the very wide range of 95% CI with a lower limit of 1.0; (ii) which was not confirmed at the evaluation of medically recorded exposure to dipyrone; and (iii) at the comparison of case and malformation control groups. In addition, different subgroups of diaphragmatic congenital abnormalities[26] did not indicate a cluster of any subgroup. Thus the major explanation for the increase of this congenital abnormality group may be again the chance effect. Nevertheless, the possible association between dipyrone and diaphragmatic congenital abnormalities can be considered as a 'signal' and it needs further studies.

The higher use of dipyrone in the mothers of malformation controls (i.e. offspring with Down's syndrome) also merits further studies.

Conclusion

The findings of this study suggest that treating pregnant women with dipyrone at usual therapeutic doses presents very little, if any, teratogenic risk to the embryo.

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