An in-vivo study on placental transfer of naproxen in early human pregnancy

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BACKGROUND: Naproxen is one of the most common non-steroidal anti-inflammatory drugs used by women of reproductive age. Naproxen is known to be teratogenic in animals. The aim of this study was to investigate the placental transfer of naproxen in the first trimester of human pregnancy, and to determine the amount of the drug in different embryonic compartments. METHODS: Twenty-eight patients who requested surgical termination of pregnancy in the first trimester were given two oral 500 mg doses of naproxen before the surgical procedure. Four biological samples, maternal venous blood, coelomic fluid, amniotic fluid and fetal tissue, were collected from each patient for drug analyses by high performance liquid chromatography. RESULTS: Naproxen was detected in all samples. The mean (± SD) concentrations were 69.5 ± 12.2 µg/ml, 6.4 ± 2.4 µg/g, 1.85 ± 1.03 µg/ml and 0.14 ± 0.11 µg/ml in maternal serum, fetal tissue, coelomic fluid and amniotic fluid respectively. The mean amniotic fluid/maternal drug ratio and fetal/maternal drug ratio were 0.002 (range 0.0005–0.0064) and 0.092 (range 0.022–0.155) respectively. There was a positive correlation between the fetal drug concentration (r = 0.59, P = 0.001), amniotic fluid drug concentration (r = 0.47, P = 0.013), amniotic fluid/maternal ratio (r = 0.536, P = 0.003) and fetal/maternal ratio (r = 0.72, P < 0.001) with advancing gestational age. CONCLUSIONS: Although naproxen can cross the placenta readily in the first trimester of human pregnancy, only a small amount was present in fetal tissues. Since there is no information on whether this small amount of naproxen would be teratogenic or not, women of reproductive age who are taking naproxen regularly should be warned of the possible fetal side-effects.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are prostaglandin synthetase inhibitors. They block the biosynthesis of prostaglandins from arachidonic acid by inhibiting cyclooxygenase, which is a crucial pathway of prostaglandin synthesis (Van den Veyver and Moise, 1993). NSAIDs are commonly prescribed worldwide, in up to 36% of in-hospital patients (Hawkins, 1998) and 11.6% of patients in general practice (Moore et al., 2000). They are useful in the treatment of conditions in all fields of medicine, including cardiology, rheumatology, orthopaedics and obstetrics and gynaecology.

The use of drugs in reproductive age women is of particular concern because of potential teratogenic effects. NSAIDs are commonly used among women of reproductive age for conditions such as dysmenorrhoea, menorrhagia, musculo-skeletal pain and tension headache (Dawood, 1993). A population-based study in Denmark revealed that 7.5% of all pregnant women had taken NSAIDs within 12 weeks before conception (Olesen et al., 1999).

Theoretically, NSAIDs may cause adverse fetal effects because of their ability to disturb the homeostasis of prostaglandins, which might result in fetal malformation (Klein et al., 1984). Indeed, aspirin and indomethacin were known to have adverse effects on human fetus and neonates if given near term (Ostensen, 1998), and teratogenic effects of the older generations of NSAID have been demonstrated in animal experiments (McGarity et al., 1981). However, similar information relating to newer generations of NSAIDs is scanty. Nielsen et al. recently reported an association between the use of NSAIDs before or during early pregnancy and a higher miscarriage rate (Nielsen et al., 2001). Whether this increase in early pregnancy loss is mediated through disturbances in early fetal development is unknown.

The teratogenicity of different drugs depends not only on their direct effect on the embryos, but also on how effectively these drugs are transported across the placenta. Naproxen has been associated with premature closure of the ductus arteriosus and severe pulmonary hypertension in infants born to mothers taking naproxen (Wilkinson et al., 1979). However, its teratogenic potential and its passage across the placenta at an early stage of pregnancy have never been studied. The objective of...
this project was to investigate the placental transfer of naproxen, a commonly used NSAID, in the first trimester of human pregnancy.

**Materials and methods**

Twenty-eight healthy pregnant women who required legal termination of pregnancy under general anaesthetic between 9 and 13 weeks gestation were recruited. These patients requested termination of pregnancy because of psychosocial reasons. They were counselled in the outpatient clinic and were only approached to participate in this study after the decision to perform termination of pregnancy had been made. Patients who had medical disease or were taking any type of medications were excluded. The study was approved by the Clinical Research Ethics Committee of the local institution. Written informed consent for this study was obtained. In all cases, ultrasound examination had confirmed a singleton pregnancy and gestational age.

Two oral doses of 500 mg naproxen (Apo-Naproxen; Apotex Inc., Toronto, Canada) were given before surgery, the first at 22:00 on the night before operation and the second 4 h before the scheduled operation time. All operations were performed between 08:30 and 13:00. Surgical termination of pregnancy was performed under general anaesthesia, using a standard protocol. Propofol and fentanyl were used for induction of anaesthesia and analgesia respectively, and anaesthesia was maintained by inhalation of 70% nitrous oxide and 1% isoflurane mixed with oxygen. A maternal venous blood sample was taken just before induction of general anaesthesia. After induction of general anaesthesia, transvaginal ultrasound examination was performed to confirm fetal viability and to measure the fetal crown-rump length. Coelocentesis and amniocentesis were performed by fine needle aspiration under ultrasound guidance transvaginally according to protocols previously reported (Lau et al., 1998). Surgical termination of pregnancy was then performed by suction curettage after the cervix was dilated with Hegar dilators to a size corresponding to the gestational age. Fetal parts were identified after the surgical procedure, washed with normal saline to remove traces of maternal blood, and collected for further analysis.

Maternal serum was separated from maternal blood by centrifugation at 1250 g for 10 min. All samples were stored at −70°C, pending further analyses. The fetus was weighed, homogenized in physiological saline and then centrifuged to clear samples before analysis. Naproxen concentrations in each of four specimens were measured by high performance liquid chromatography with UV detection. The minimal detection limits of the assay were 10 ng/ml for amniotic fluid, coelomic fluids and fetal tissue samples, and 20 ng/ml for maternal serum samples. The inter-batch assay of naproxen was reproducible and precise, with a coefficient of variation of 4.94%. Naproxen metabolites in the samples were not analysed.

Drug concentrations in serum, amniotic and coelomic samples were expressed in drug per unit volume (per ml), while those in fetal tissue were expressed as drug per unit weight (per g). Since the density of fetal tissue should be >1 g/ml, the numerical value of the drug concentration should be higher when expressed in µg/ml than when expressed in µg/g. Therefore, interpretation of ratios involving fetal tissue drug concentrations should be cautious.

Wilcoxon signed ranks test was used to determine the differences in drug concentration between these samples, and Pearson correlation analysis was used for the rest of the tests.

**Results**

The mean gestational age of pregnancy was 10.5 weeks (range 9.1–13.3). Biological samples were collected on average 13.6 (range 12.4–15.5; median 13.7; SD 0.78) and 4.5 h (range 3.8–5.3; median 4.5; SD 0.43) after the first and second dose of naproxen respectively.

Naproxen was detected in all maternal serum, coelomic fluid, amniotic fluid and fetal tissue samples. Maternal serum naproxen concentrations (mean ± SD) ranged from 25.3 to 88.15 µg/ml (69.5 ± 12.2). Fetal tissue drug concentration ranged from 1.8 to 10.6 µg/g (6.4 ± 2.4). Coelomic fluid concentration ranged from 0.42 to 5.92 µg/ml (1.85 ± 1.03) and amniotic fluid drug concentration from 0.03 to 0.53 µg/ml (0.14 ± 0.11). There were highly significant differences between drug concentrations in maternal serum and fetal tissue; fetal tissue and coelomic fluid; and coelomic fluid and amniotic fluid; (P < 0.001). Figure 1 shows the relationship between naproxen concentrations in different samples and gestational age. There were significant correlations between gestational age and drug concentration (r = 0.59, P = 0.001), and amniotic fluid drug concentration (r = 0.47, P = 0.013), but not between gestational age and coelomic fluid (r = −0.1).

Fetal/maternal naproxen ratio was calculated by dividing the fetal tissue drug concentration by the corresponding maternal serum concentration. The mean (range) ratio was 0.092 (0.022–0.155). The mean coelomic fluid/maternal ratio, mean amniotic fluid/maternal ratio and mean amniotic fluid/fetal drug ratio were calculated similarly, and were 0.028 (0.009–0.080), 0.002 (0.0005–0.0064) and 0.024 (0.006–0.080) respectively. There was a direct correlation between fetal/maternal ratio and gestational age (r = 0.72, P < 0.001; Figure 2), and between gestational age and amniotic fluid/maternal ratio (r = 0.536, P = 0.003). However, neither

Figure 1. A scatter plot of naproxen concentration in different biological samples against gestational age. There was significant direct relationship between both fetal tissue (r = 0.59, P = 0.001), and amniotic fluid (r = 0.47, P = 0.013) drug concentration with gestational age.
anaesthesia (Jorgensen et al., 1988; A.J. Jauniaux et al., 1996, 1998; Shannon et al., 1998) and antibiotics (Dekel et al., 1980; Nau et al., 1981; Heisterberg, 1984; Karhunen, 1984; Jorgensen et al., 1987). All these studies used similar methodology, which was to study drug concentration in different fetal compartments after the maternal administration of a drug before termination of pregnancy. By direct sampling of the drug concentration in different compartments of the embryo, the drug concentration and the transfer ratio could be determined. This model is closest to the real situation (Jauniaux and Gulbis, 2000a), and was therefore adopted for the current study.

Our results have confirmed that naproxen crosses the first trimester human placenta. Naproxen was found in all samples collected from all three embryonic compartments, namely the amniotic cavity, the extra-embryonic cavity and the fetus. However, there were significant differences between drug concentrations among these compartments, the highest concentration being found in the fetus, and the lowest in the amniotic cavity. This gradient of drug concentration is probably due to different mechanisms involved in placental drug transfer in early pregnancy as proposed by Jauniaux and Gulbis (2000b). On the other hand, amniotic fluid is a combination of transudate from fetal skin and transfer from coelomic fluid. Drugs enter the fetal tissue either directly through villous tissue or retrieval by secondary yolk sac from extracoelomic fluid (Gulbis et al., 1998).

Most drugs cross the placenta by passive diffusion, which is affected by molecular weight, liposolubility, ionization and protein binding (Garland, 1998). Naproxen is a highly liposoluble molecule with a low molecular weight of 230 Daltons. These chemical characteristics enable naproxen to cross biological membranes easily. A significant amount of naproxen was detected in the fetal tissue and less in the extracoelomic cavity, suggesting that naproxen crosses villous tissue more readily than the chorionic leaf. At therapeutic concentrations, >99% of naproxen is tightly bound to albumin (Allison et al., 1985). Thus, this explained why the coelomic and fetal tissue naproxen concentration attained was only 8–15% of the corresponding serum concentration. Since the amniotic membrane has a lower rate of drug transfer than the chorionic leaf (Jauniaux and Gulbis, 2000b), the naproxen concentration in amniotic fluid was lowest among all the fetal compartments.

We have found that naproxen concentration increased in both the amniotic fluid and fetal tissue with advancing gestational age. This increase in transfer might be due to the development and maturation of villous tissue, which improves the maternal–fetal transfer into these two compartments. The lack of gestation dependency of naproxen concentration in coelomic fluid in our study further supports the concept that the transfer of naproxen is independent of chorionic villi, but instead occurs via ultra-filtratation through the chorionic laeve.

Our results show that the placental transfer of naproxen is significantly different from that of diclofenac (Siu et al., 2000), which is another NSAID. In the diclofenac study, the fetal concentration was close to the maternal concentration, with a mean fetal/maternal ratio of 1.05. In contrast, the mean fetal/maternal ratio of naproxen was 0.092, which was only 10% of the maternal drug concentration. Therefore, the fetal naproxen concentration increases with gestational age, while fetal diclofenac decreases with gestational age. These differences may represent different placental transfer mechanisms of these two drugs. Our observations suggest that diclofenac has a higher potential to induce fetal effects because of a much higher rate of fetal transfer.

Previous studies on the placental transfer of propofol (Jauniaux et al., 1998) and fentanyl (Cooper et al., 1999) yielded somewhat different results. Neither propofol nor fentanyl was detectable in any coelomic fluid or amniotic fluid samples collected within 30 min after a single i.v. injection.
Jauniaux and Gulbis hypothesized that coelomic fluid has a slow turnover in nature, therefore it may take longer for a drug to reach the coelomic cavity and thus the amniotic cavity (Jauniaux and Gulbis, 2000b).

In this study, subjects were prescribed two doses of naproxen before pregnancy termination. This regime was different from the propofol and fentanyl studies in which only a single dose before pregnancy termination. This regime was different from (Jauniaux and Gulbis, 2000b).

In conclusion, naproxen crosses the placenta readily and the transfer ratio increases with gestation. Though the fetal/maternal ratio of naproxen is small, we have no information on whether this small amount of naproxen would cause any adverse effects on the developing human fetus. With the current evidence from rat embryo culture that naproxen could induce cleft palate (Montenegro and Palomino, 1990), naproxen should be regarded as a potential teratogenic drug until proven otherwise. On the other hand, the much lower rate of placental transfer of naproxen compared with diclofenac suggests that the former is less likely to induce teratogenicity. Nonetheless, we believe that women of reproductive age who are taking NSAIDs regularly should be warned of the possible fetal side-effects.

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References


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