RIITTA OJALA

Effects of Perinatal Indomethacin Treatment on Preterm Infants

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ACADEMIC DISSERTATION

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for public discussion in the small auditorium of Building K,
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, referred to in the text by their roman numerals I-IV. In addition, previously unpublished data are also presented.

I Tammela O, Ojala R, Iivainen T, Lautamatti V, Pokela M-L, Janas M, Koivisto M, Ikonen S. Short versus prolonged indomethacin therapy for patent ductus arteriosus in preterm infants. J Pediatr 1999;134:552-7.

II Ojala R, Ikonen S, Tammela O. Perinatal indomethacin treatment and neonatal complications in preterm infants. Eur J Pediatr 2000,159:153-5.

III Ojala R, Ruuska T, Karikoski R, Ikonen R.S, Tammela O. Gastroesophageal endoscopic findings and gastrointestinal symptoms in preterm neonates with and without perinatal indomethacin exposure. In press (J Pediatr Gastoenterol Nutr).

IV Ojala R, Ala-Houhala M, Ahonen S, Harmoinen A, Turjanmaa V, Ikonen R.S, Tammela O. Renal follow-up of premature infants with and without perinatal indomethacin exposure. In press (Arch Dis Child).

ABBREVIATIONS

BP	blood pressure
BPD	bronchopulmonary dysplasia
CI	confidence interval
COX	cyclo-oxygenase
DA	ductus arteriosus
GFR	glomerular filtration rate
GI	gastrointestinal
IVH	intraventricular haemorrhage
NEC	necrotizing enterocolitis
OR	odds ratio
PDA	patent ductus arteriosus
PG	prostaglandin
RDS	respiratory distress syndrome
SD	standard deviation
SGA	small for gestational age
Tx	thromboxane
UAC	umbilical artery catheter

INTRODUCTION

Indomethacin, a prostaglandin (PG) and thromboxane (Tx) synthetase inhibitor, has been used in perinatal medicine by both obstetricians and neonatologists. It has been administered antenatally for the prevention of preterm labor since the early 1970s (Zuckerman et al. 1974) and for the treatment for polyhydramnios since the 1980s (Cabrol et al. 1987). Postnatal indomethacin use in closing a patent ductus arteriosus (PDA) in a premature infant was first described in 1976 (Friedman et al. 1976, Heyman et al. 1976) and the effectiveness of indomethacin administration during the first days of life in preventing PDA and intraventricular haemorrhage (IVH) in infants born prematurely was suggested a few years later (Merritt et al. 1981, Mahony et al. 1982, Setzer et al. 1984).

During the last two decades several authors have pondered safety of indomethacin use. Significant adverse effects, including isolated bowel perforations, necrotizing enterocolitis (NEC), bleeding tendency and renal dysfunction have been described after maternal (Vanhaesebrouck et al. 1988, Norton et al. 1993) and postnatal (Seyberth et al. 1983b, Rennie et al. 1986, Bandstra et al. 1988, Grosfeld et al. 1996) indomethacin exposure in premature infants. Antenatal indomethacin administration has also been connected with an increased risk of persistent pulmonary hypertension in newborns (Levin et al. 1979, Van Marter et al. 1996), respiratory distress syndrome (RDS) (Van Overmeire et al. 1998), bronchopulmonary dysplasia (BPD) (Eronen et al. 1994, Van Overmeire et al. 1998) and IVH (Norton et al. 1993, Souter et al. 1998), although contrary opinions have also been put forward (Gardner et al. 1996, Vermillion and Newman 1999).

The safety of selective cyclo-oxygenase (COX)-2 inhibitors and their usefulness in the prevention of preterm delivery has recently been suggested (Sadovsky et al. 2000), but at least neonatal renal dysfunction has been shown even after COX-2 inhibition (Peruzzi et al. 1999). Furthermore, ibuprofen, another PG synthetase inhibitor, has been an equal constrictor of the ductus arteriosus

(DA) with lesser renal side-effects than indomethacin in premature infants (Mosca et al. 1997, Pezzati et al. 1999, Van Overmeire et al. 2000). However, its safety in most immature infants, and its long-term effects call for further evaluation. It would thus appear that indomethacin treatment retains its place in perinatal medicine.

Despite active investigation of indomethacin effects on premature infants, there is lack of data concerning the effects of combined ante- and postnatal indomethacin exposure on infants born prematurely and only little information as to the long-term effects of perinatal indomethacin administration. The purpose of the present study was to compare two postnatal indomethacin administration regimens for PDA closure, to ascertain the effects of antenatal, postnatal and combined ante- and postnatal indomethacin exposure on the endoscopic findings in the upper gastrointestinal tract and on the morbidity of infants during their primary hospitalization, and to evaluate long-term renal findings in early childhood in cases with and without perinatal indomethacin exposure, born at <33 weeks' gestation.

REVIEW OF THE LITERATURE

1. Pharmacology and metabolism of indomethacin

Indomethacin is a nonsteroidal anti-inflammatory drug first introduced in 1963 (Shen et al. 1963). The structural formula of indomethacin is that of a methylated indole derivative. Indomethacin inhibits COX enzymes and prevents the formation of prostaglandin PGE_2, $PGF_{2\alpha}$, PGD_2, prostacyclin (PGI_2) and TxA_2 from arachidonic acid (Smith and Dewitt 1996) (Figure 1).

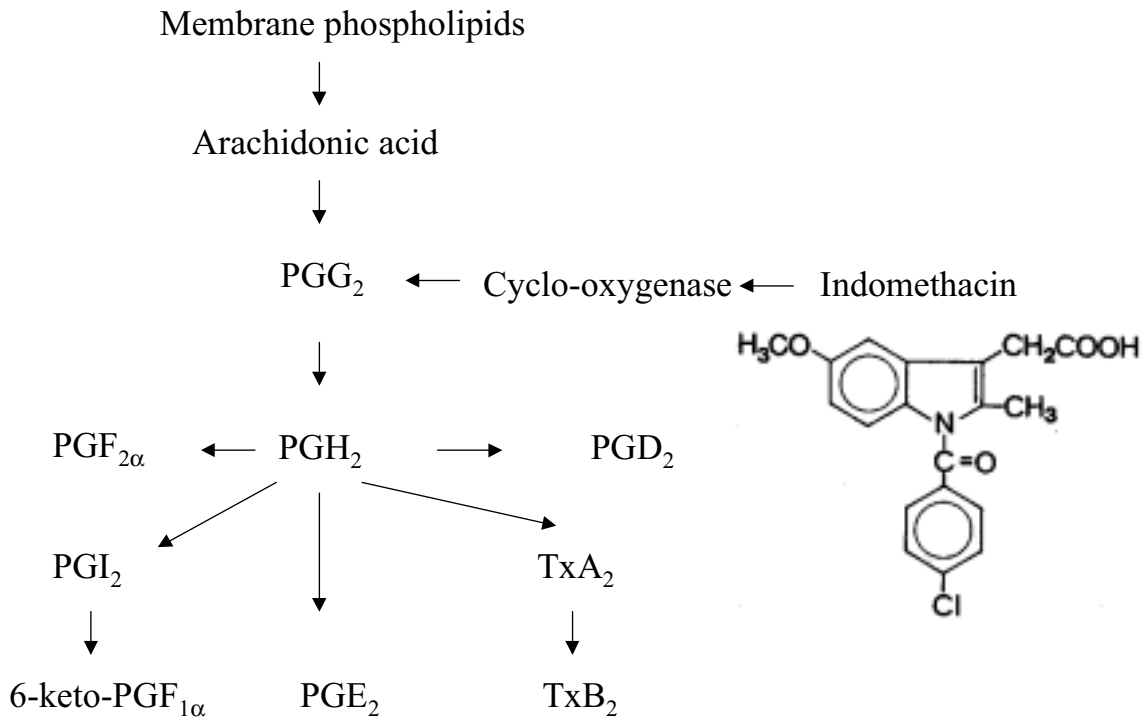

Figure 1. Biosynthesis of prostaglandins and thromboxanes via the cyclooxygenase pathway and the structural formula of indomethacin.

There are two types of COX isoenzymes: COX-1 and COX-2, and indomethacin has an inhibitory impact on both of them (Mitchell et al. 1994). The general conception has been that COX-1 is expressed in most tissues and that PGs contributing to homeostatic functions are derived from it, whereas COX-2 is induced in inflammatory cells and is the enzyme which produces prostanoid mediators for inflammation. The anti-inflammatory action of indomethacin has been thought to be related to COX-2 inhibition and the undesirable effects of the drug to COX-1 inhibition (Rang et al. 1995). There is evidence, however, that COX-2 is expressed in many tissues, including DA and the kidney, where it has physiological functions (Clyman et al. 1999a, Wallace 1999). There is also evidence suggesting an important COX-1-mediated component in inflammation (Wallace 1999).

In adults, indomethacin is over 90% bound to plasma proteins, mainly to albumin. It is metabolized through hepatic conjugation with glucuronic acid, O-demethylation and N-deacylation to inactive metabolites (Insel 1995). Possibly as a result of enterohepatic cycling the plasma half-life of indomethacin is variable, ranging between 3 and 11 hours (Alvan et al. 1975, Kwan et al. 1976). When administered to pregnant women orally, rectally or vaginally, it crosses the human placenta easily throughout gestation, the mean maternal/foetal serum ratio being up to 0.97 (Moise et al. 1990, Lampela et al. 1999, Abramov et al. 2000).

In premature infants, plasma concentrations six hours after a single intravenous dose of indomethacin can vary about 5-fold (Brash et al. 1981). The protein-binding capacity of indomethacin is similar to that in adults (Bhat et al. 1979). The elimination half-life appears to be prolonged, even up to 90 hours (Vert et al. 1980) and decreases with advancing gestational and postnatal age (Bhat et al. 1979, Thalji et al. 1980).

2. Use of indomethacin in perinatology

2.1. Antenatal use

2.1.1. Prevention of preterm labor

Local production of PGE_2 and $F_{2\alpha}$ seems to have an important role in the initiation of labor (Brennand et al. 1995, Sugimoto et al. 1997). Sadovsky and associates (2000) reported increased COX-2 expression and two- to five-fold higher PGE_2 levels in amnion samples derived from women in both preterm and term labor compared with samples from nonlaboring women, levels in samples obtained from term labor being higher than in those from preterm labor. It is thus possible that the uterus is more responsive preterm than at term to stimulation by PGs (Sadovsky et al. 2000).

Since the early 1970s indomethacin has been used as a tocolytic agent (Zuckerman et al. 1974). The first randomized, double blind, placebo-controlled trial to establish the efficacy of indomethacin in delaying labor was reported in 1980 (Niebyl et al. 1980). Its ability to inhibit myometrial contractility seems to derive from inhibition of PG synthesis and possibly blockade of the Ca^{2+} channel current (Sawdy et al. 1998).

2.1.2. Treatment of polyhydramnios

Another indication for the use of indomethacin during pregnancy is in the treatment of polyhydramnios (Cabrol et al. 1987). Major sources of amniotic fluid production are foetal urination and egress of foetal lung fluid, whereas foetal swallowing and intramembraneous absorption across the foetal surface of the placenta are responsible for removal of amniotic fluid (Gilbert and Brace 1989, Cunningham et al. 1997). Indomethacin reduces the amniotic fluid volume via reduction of foetal urine production (Kirshorn et al. 1988) and enhances fluid resorption by increasing foetal breath (Hallak et al. 1992).

2.1.3. Safety

Indomethacin has been reported to be effective, inexpensive and mostly well tolerated by the mother (Morales et al. 1989, Kurki et al. 1991). Maternal side-effects are usually minimal, the most common being gastrointestinal, including nausea, heartburn and vomiting, or neurological, including headache, vertigo and jitteriness (Besinger et al. 1991, Kurki et al. 1991). Indomethacin can also prolong the maternal bleeding time and may increase the risk of postpartum haemorrhage (Reiss et al. 1976, Lunt et al. 1994). Prolonged indomethacin therapy can cause oligohydramnios and impairment of maternal renal function (Carmona et al. 1993).

Compared with beta-sympathomimetic agents, indomethacin shows at least equal inhibition of uterine contractions and has been associated with fewer maternal side-effects (Morales et al. 1989, Besinger et al. 1991, Kurki et al. 1991).

2.2. Postnatal use

2.2.1. Treatment of the patent ductus arteriosus

The effectiveness of indomethacin in closing PDA in premature infants was first reported in 1976 (Friedman et al. 1976, Heymann et al. 1976). Indomethacin was initially administered per rectum or orally, but intravenous administration has proved simpler and more effective (Vert et al. 1980). The intravenous single dose ranges from 0.1 mg/kg to 0.4 mg/kg and the duration of the infusion varies from bolus treatment to a 15-30-minute or even to continuous, 36-hour infusion (Gal et al. 1991, Hammerman et al. 1995). The regimens most commonly adopted include a short 1-3 dose schedule with doses given at 12- to 24-hour intervals or prolonged 6-7-day therapy with doses administered at eight- to 24-hour intervals (Hammerman and Aramburo 1990, Thalji et al. 1980).

2.2.2. Prophylactic use

Indomethacin has been used prophylactically for the prevention of PDA and IVH in infants born preterm. In both indications treatment is initiated during the first 24 hours of life, the intravenous single dose being 0.1 or 0.2 mg/kg and regimens varying from one to 5 doses at 12- or 24-hour intervals (Fowlie 1996).

3. Effects of antenatal use on the foetus and newborn

3.1. The foetus

3.1.1. Effects on the ductus arteriosus

Patency of the DA is an active state maintained by the action of PGs, PGE_2, PGI_2 and its metabolite 6-keto-$PGF_{1\alpha}$ being the major mediators of ductus dilatation (Clyman et al. 1978, Kääpä 1984, Hammerman et al. 1986). Antenatal indomethacin administration inhibits both local ductal and systemic synthesis of foetal PGs (Pace-Asciak and Rangaraj 1978, Mäkilä et al. 1983, Clyman et al. 1999a, Takahashi et al. 2000). In Doppler ultrasound studies maternal indomethacin treatment has been shown to cause constriction of the foetal DA in as many as 86% of cases (Eronen 1993), in some cases connected with tricuspid valve regurgitation as first sign of developing cardiac failure (Eronen 1993, Van den Veyver et al. 1993). Ductal constriction has been independent of foetal serum indomethacin levels and can occur even after a single dose of the drug (Van der Veyver et al. 1993, Räsänen and Jouppila 1995). This effect can be seen as early as at 24 weeks of gestation, but the foetal ductus would appear to become more reactive to indomethacin with increasing gestational age, the maximum effect being seen at 31 or 32 weeks (Eronen 1993, Moise 1993, Vermillion et al. 1997). The constriction is apparently reversible, normal flow velocities returning after discontinuation of indomethacin treatment (Eronen 1993, Räsänen and Jouppila 1995, Vermillion et al. 1997).

3.1.2. Pulmonary effects

PGs appear to be of importance in the control of pulmonary vascular resistance, $PGF_{2\alpha}$ acting as a pulmonary vasoconstrictor, PGI_2 PGE_2 and PGE_1 as pulmonary vasodilatators and PGD_2, depending on dose and age, as a pulmonary vasoconstrictor or vasodilator (Lock et al. 1980a, Cassin 1987).

In animal studies antenatal indomethacin treatment has increased foetal mean pulmonary arterial blood pressure and the ratio of mean pulmonary arterial to

mean systemic arterial blood pressure. These effects were postulated to be due to constriction of the foetal DA (Levin et al. 1979). Furthermore, an increase in medial smooth muscle mass in the foetal pulmonary vessels after maternal indomethacin treatment has been suggested (Levin et al. 1979, Harker et al. 1981). In human studies maternal indomethacin therapy increases foetal pulmonary arterial vascular impedance even without constriction of the DA. Also, it would seem that after 26 weeks' gestation the human foetus is able to regulate pulmonary arterial vascular tone in response to increased pulmonary arterial pressure caused by ductus constriction (Räsänen et al. 1999). Indomethacin has been found to reduce surfactant protein-A mRNA levels, alveolar lumen size and lamellar body volume density of human foetal lung in vitro (Acarregui et al. 1990).

3.1.3. Cerebral effects

In animal studies, indomethacin has reduced foetal cerebral blood flow and improved cerebral autoregulation (Hohimer et al. 1985, Van Bel et al. 1995). Skarsgard and colleagues (1999) found in a Doppler study that maternal administration of indomethacin produced a trend toward decreased foetal carotid blood flow, flow variability and increased carotid resistance. In human foetuses, Mari and associates (1989) reported antenatal indomethacin exposure to lower the pulsatility index of the middle cerebral artery if ductal constriction was associated with tricuspid insufficiency in foetuses between 25-33 weeks' gestation. In contrast, Parilla and coworkers (1997) found no differences between the resistance index of the foetal middle cerebral artery measured during and after tocolysis with indomethacin. However, they measured neither ductal constriction nor tricuspid regurgitation, which may have influenced their results (Parilla et al. 1997).

3.1.4. Renal effects

Major sites of renal PG synthesis are the arteries, arterioles and glomeruli in the cortex, cortical and medullary collecting tubules and medullary interstitial cells. The proximal tubule, the loop of Henle and the connecting segment of the distal tubule show only little ability to produce PGs. PG production and release in the cortex maintains glomerular filtration and blood flow. Tubular PGs modulate

water and electrolyte transport and medullary PG production maintains the blood flow in the medulla (Schlondorff 1986). In addition to these direct effects, PGs also exert indirect effects through interaction with other systems, including the renin-angiotensin system (Guignard et al. 1991).

In animal studies foetal indomethacin exposure has been found to be associated with a decrease in foetal urine PGE and $PGF_{2\alpha}$, levels, urine output and plasma renin activity and with an increase in urinary sodium and chloride excretion, and in urine osmolality (Matson et al. 1981, Walker et al. 1992). Indomethacin and other PG synthesis inhibitors reduce the foetal renal blood flow and increase renal vascular resistance (Matson et al. 1981).

In human foetuses <33 weeks gestation, urine output has been observed to decline significantly as early as 5 hours after maternal indomethacin administration, being low throughout therapy and normalizing within 24 hours after therapy (Kirshon et al. 1988). Antenatal indomethacin administration has also led to significant oligohydramnios and foetal hydrops among patients treated (Mogilner et al. 1982, Vanhaesebrouck et al. 1988). No changes in foetal renal pulsatility index values after antenatal indomethacin administration have been noted, at least not during the first 24 hours of therapy (Mari et al. 1990).

3.1.5. Gastrointestinal effects

In animal studies indomethacin has been shown to inhibit PG production in the foetal mesenteric arteries in vivo (Shaul et al. 1992).

3.1.6. Platelet function

PGs and TxA_2 regulate platelet function, TxA_2 being a potent inductor of platelet adhesion and aggregation. PGI_2 inhibits platelet aggregation and thus counters the effects of TxA_2 (Gorman 1979). Indomethacin has been shown to block TxB_2, the stabile TxA_2 metabolite synthesis in foetal animals and in human platelets in vitro (Mäkilä et al. 1983, Kunievsky and Yavin 1992).

3.2. The newborn

3.2.1. Effects on the ductus arteriosus

In premature lambs a ductus initially constricted in the uterus has limited ability to contract actively in response to oxygen or indomethacin, this phenomenon being more clearly seen near term (Clyman et al. 1985). Indomethacin tocolysis, especially drug administration within 48 hours before delivery has been claimed to increase the incidence of asymptomatic and symptomatic PDA in premature infants (Norton et al. 1993, Hammerman et al. 1998, Souter et al. 1998), but also reports to the contrary have been published (Eronen 1993). A lesser responsiveness to therapeutic indomethacin treatment and an increased need for surgical ligation of the PDA after prenatal indomethacin exposure has been suggested (Norton et al. 1993, Eronen et al. 1994, Hammerman et al. 1998, Van Overmeire et al. 2000), and the association between antenatal indomethacin exposure and symptomatic PDA seems to increase with increasing maturity of the infant (Norton et al. 1993).

3.2.2. Pulmonary effects

3.2.2.1. Persistent pulmonary hypertension in the newborn

Maternal consumption of indomethacin or other nonsteroidal anti-inflammatory drugs during pregnancy has been associated with an increased risk of persistent pulmonary hypertension in the newborn (Levin et al. 1979, Van Marter et al. 1996). Intrauterine constriction of the DA and/or direct pulmonary vasoconstriction might cause foetal pulmonary arterial hypertension and give rise to media thickening in the pulmonary arteries, this bringing about a postnatal fall in pulmonary vascular resistance (Levin et al. 1978, 1979, Wild et al. 1989). However, several other investigators have not confirmed such an association (Besinger et al. 1991, Eronen et al. 1994, Vermillion and Newman 1999).

3.2.2.2. Respiratory distress syndrome

There are contradictory reports regarding the effects of maternal indomethacin treatment on RDS incidence, and different protocols of antenatal corticosteroid treatment and indomethacin use may have influenced results. In trials matched for gestation and antenatal corticosteroid administration, both an increased (Van Overmeire et al. 1998) and a decreased (Gardner et al. 1996) incidence of RDS and need for surfactant use have been reported in premature infants after antenatal indomethacin exposure. Maternal indomethacin treatment initiated five or less days before delivery has also been held to increase the incidence of RDS among infants (Eronen et al. 1994). Again, however, several other studies report no influence of antenatal indomethacin exposure on RDS or need for surfactant use (Morales et al. 1989, Norton et al. 1993, Panter et al. 1999).

3.2.2.3. Bronchopulmonary dysplasia and pneumothorax

Effects of antenatal indomethacin treatment on the incidence of pneumothorax and BPD have rarely been reported in premature infants and in all reports BPD has been mainly diagnosed according to the criteria of Bancalari, i.e. at the age of 28 days (Bancalari and Gerhardt 1986).

Eronen and colleagues (1994) compared indomethacin and nylidrin tocolysis in a randomized trial and found an increased incidence of BPD in 42 infants with prenatal indomethacin exposure compared to 45 infants exposed to nylidrin. In an retrospective study of 76 infants, mostly delivered within 10 hours of exposure, an association between antenatal indomethacin treatment and an increased incidence of BPD was found (Van Overmeire et al. 1998). In a placebo-controlled study of 34 infants BPD, diagnosed at 36 weeks' postconceptional age, was over twice as common in the indomethacin group as in the control group, the difference, however, not being statistically significant (Panter et al. 1999). In contrast, other investigators have seen no differences in BPD incidence between infants with and without indomethacin exposure even with an interval of 48 hours

or less between the last dose of the drug and delivery (Vermillion and Newman 1999, Norton et al. 1993).

Indomethacin treatment has not been observed to affect the incidence of pneumothorax in antenatally exposed infants (Van Overmeire et al. 1998).

3.2.3. Cerebral effects

3.2.3.1. Intraventricular haemorrhage

There are controversial reports regarding the effects of antenatal indomethacin on the incidence of IVH in premature infants. A retrospective study of 124 infants by a group under Gardner (1996) and a case-control analysis of 225 infants by Vermillion and Newman (1999) found no differences in the incidence of IVH between the study and control groups in premature infants born within two days of antenatal indomethacin exposure. A small randomized study of 34 infants found no differences in grade I-IV IVH incidence in infants born at 30 weeks' gestation or less exposed to either indomethacin or placebo (Panter et al. 1999). Convergent results are also seen in studies comparing indomethacin to other tocolytic agents (Besinger et al. 1991, Eronen et al. 1994, Parilla et al. 1997). On the other hand, Iannucci and associates (1996) suggested an increased risk of grade III-IV IVH among 22 infants <800g receiving dual tocolytic therapy with indomethacin and magnesium sulfate compared with 34 receiving magnesium sulfate therapy alone. There are also retrospective studies reporting an increased risk of grade I-II and III-IV IVH (Souter et al. 1998) and on the other hand grade II IVH (Norton et al. 1993) in infants born at < 31 weeks' gestation and within 48 hours of maternal indomethacin exposure.

3.2.3.2. Periventricular leukomalacia

In a study of 159 infants at <30 weeks' gestation, a higher incidence of polycystic periventricular leukomalacia has been found in cases with prenatal indomethacin exposure than in those without (Baerts et al. 1990). A randomized placebo-controlled study of 34 infants, again, showed no such connection (Panter et al. 1999).

3.2.4. Renal effects

Transient oliguria, oedema, metabolic acidosis, low serum sodium and increased serum potassium and creatinine levels have been reported in premature infants both a few days and several weeks after antenatal indomethacin adminstration (Vanhaesebrouck et al. 1988, Kaplan et al. 1994). Also sporadic cases of persistent lethal anuria has been described in neonates after prenatal indomethacin exposure (Van der Heijden et al. 1994), although no differences have been found in the incidence of anuria between infants born at <33 weeks gestation with and without antenatal indomethacin exposure (Gardner et al. 1996, Vermillion and Newman 1999). Furthermore, maternal indomethacin treatment has been associated with a reduction in glomerular filtration rate (GFR) and increased urine osmolarity in the first days of life in preterm infats (Van der Heijden et al. 1988, Van den Anker et al. 1994). No dose effect of antenatal administration on newborn renal function has been noted, but lower urine output and higher serum creatinine concentrations during the first three days have been suggested in infants born at <31-32 weeks' gestation if the mothers have received their last dose of indomethacin within 48 hours before delivery (Van der Heijden et al. 1988, Norton et al. 1993, Van den Anker et al. 1994).

Ultrasound examination performed after several weeks' intrauterine indomethacin exposure has revealed enlarged kidneys, increased echogenicity and poor renal corticomedullary differentiation in premature infants (Kaplan et al. 1994, Buderus et al. 1993). Also histopathological changes, including abnormal tubular differentiation, variable tubular dilatation, small, immature glomeruli with glomerular cysts and interstitial fibrosis have been described after prenatal indomethacin exposure (Kaplan et al. 1994, Van der Heijden et al. 1994).

3.2.5. Gastrointestinal effects

3.2.5.1. Necrotizing enterocolitis

An increased incidence of confirmed NEC, defined as pneumatosis intestinalis or bowel perforation, has been described after antenatal indomethacin exposure in a retrospective study of 114 infants born at < 31 weeks' gestation (Norton et al.

1994). Delivery within 120 hours (Eronen et al. 1994) or, in infants with a birthweight <1500 g within 24 hours after the initiation of maternal indomethacin treatment and at least 48 hours' duration of antenatal exposure have been presented as risk factors underlying confirmed NEC (Major et al. 1994). However, case control studies of 225 infants (Vermillion and Newman 1999) and 120 infants (Parilla et al. 2000) have brought out no significant differences in the incidence of NEC between controls and cases exposed antenatally to indomethacin, although delivered within 48 hours of maternal treatment.

3.2.5.2. Isolated bowel perforation

Sporadic cases of isolated intestinal perforation without necrosis have been reported after prenatal indomethacin exposure in preterm infants (Vanhaesebrouck et al. 1988, Norton et al. 1993, Feijgin et al. 1994). These focal perforations have usually been ileal, but a duodenal perforation has also been reported (Vanhaesebrouck et al. 1988, Eronen et al. 1994).

3.2.6. Bleeding tendency

Absence of platelet aggregation has been reported in premature infants born after prenatal indomethacin administration (Vanhaesebrouck et al. 1988), but no significant effects on platelet count, prothrombin time or activated partial thromboplastin time have been shown (Vanhaesebrouck et al. 1988, Morales et al. 1989). GI bleeding in preterm infants after antenatal indomethacin administration has also been suggested (Vanhaesebrouck et al. 1988).

4. Effects of postnatal use on the newborn

4.1. Closure and reopening of the ductus arteriosus

Prior to birth 90% of the right ventricular output flows into the descending aorta through the DA and only 10% enters the pulmonary circulation. After birth ductal closure occurs in two stages. Shortly after birth functional constriction of the ductus begins, with subsequent anatomic closure. Alterations in oxygen tension

and PG levels are major factors influencing the former. Increased oxygen tension stimulates ductal constriction and increases PGE_2 production in the DA (Noel and Cassin 1976, Rabinovitch et al. 1989). In contrast, there are conflicting data regarding the oxygen tension effects on PGI_2 and its metabolite 6-keto-$PGF_{1\alpha}$ production (Rabinovitch et al. 1989, Stuart et al. 1984).

In preterm infants without RDS the functional closure of the DA usually occurs within three days (Evans and Archer 1990). However, acidosis, absence of an increase in oxygen tension or a decreased ductal contractile response to oxygen can maintain the patency of the DA (Noel and Cassin 1976, Archer 1999). High circulating levels of PGI_2 metabolite 6-keto-$PGF_{1\alpha}$ would appear to be correlated with clinically significant PDA in preterm infants (Hammerman et al. 1986, Kluckow et al. 1999), while circulating levels of PGE_2 and TxA_2 have been similar in preterm patients with and without PDA (Clyman et al. 1980, Kuehl et al. 1986).

Inhibition of PG synthesis can be achieved in preterm infants even with low plasma concentrations of indomethacin, the synthesis returning about five days after indomethacin has been discontinued (Rennie et al. 1986). In an animal trial, dilatation of the DA after infusion and re-elevation of the dilator PGE_2 level was directly related to the degree of ductal shunt before infusion (Clyman et al. 1983). Ductuses of immature lambs have been more prone to re-dilate after initial ductus constriction when compared with that in more mature lambs (Clyman et al. 1985). Furthermore, Clyman and colleagues (1999b) found that functional ductal constriction causes the development of vessel wall hypoxia with increased expression of vascular endothelial cell growth factor and proliferation of endothelial cells in newborn baboons. These changes seemed to fail to develop in most immature baboons although their ductus was functionally closed (Clyman et al. 1999b). It is thus possible that failure to develop ductal hypoxemia together with residual luminal flow of the DA, immaturity and restored PG production after indomethacin treatment may increase the risk of clinical reopening of the DA and failure of anatomic ductal closure (Weiss et al. 1995, Clyman et al. 1999b, Narayanan et al. 2000).

4.1.1. Treatment of the patent ductus arteriosus

Postnatal short-term intravenously administered indomethacin has proved ineffective in closing the ductus in 21% of 421 infants with birthweight <1750g and symptomatic PDA, a relapse occurring in 26% of responders (Gersony et al. 1983). In randomized controlled studies of 70 (Rhodes et al. 1988) and 121 infants (Rennie and Cooke 1991), where the diagnosis of PDA was clinical, without echocardiographic confirmation, a prolonged regimen of indomethacin correlated with a higher response rate and a lower reopening rate than a short protocol (Rhodes et al. 1988, Rennie and Cooke 1991). In an uncontrolled study of 148 infants with birthweight <1500g and a haemodynamically significant PDA, 90% response and only 3% recurrence rate was achieved with six days of indomethacin therapy (Kumar and Yu 1997). Response of the ductus does not correlate well with the plasma indomethacin concentration (Alpert et al. 1979, Ment et al. 1988), although controversial data have also been presented (Brash et al. 1981, Seyberth et al. 1983a, Gal et al. 1990). Rapid metabolism of the drug, low gestational age, high postnatal age and antenatal indomethacin exposure may be associated with a lower success rate in initial ductal closure with indomethacin (Brash et al. 1981, Firth and Pickering 1980, Norton et al. 1993, Trus et al. 1993, Van Overmeire et al. 2000). Low gestational age is also connected with reopening of the DA (Weiss et al. 1995).

4.1.2. Prophylactic treatment

The association between prophylactic indomethacin treatment administered during the first 24 hours after birth and a decreased incidence of PDA in premature infants is well established (Fowlie 1996). In infants with birthweight <1251g the incidence of PDA has been 34-54% among placebo-treated infants and 10-28% with prophylactic treatment at five days of age (Ment et al. 1988, 1994a). Also the incidence of symptomatic PDA has decreased after prophylactic treatment in infants with a birthweight <1301g (Bandstra et al. 1988) and <1501g (Krueger et al. 1987). However, even when managed prophylactically, the rate of ductus reopening is high in most immature infants (Narayanan et al. 2000).

4.2. Pulmonary effects

There is scant information as to effects of postnatal indomethacin treatment on pulmonary haemodynamics in newborns. In 2-12-week-old lambs pulmonary vasoconstriction has occurred after an indomethacin dose of only 0.01 mg/kg. However, the lung seems to adapt to indomethacin and a therapy of three days no longer altered base-line pulmonary tone (Lock et al. 1980b). In infants born at under 34 weeks' gestation only a small decrease in pulmonary artery peak and mean blood velocity with a decrease in ductal velocities after single-dose indomethacin administration has been seen (Benders et al. 1999).

4.2.1. Respiratory distress syndrome

Prophylactic indomethacin exposure has had no effect on the need for surfactant, the duration of oxygen supplementation or ventilator treatment required in premature infants (Hanigan et al. 1988, Bada et al. 1989, Ment et al. 1994a). Placebo-controlled studies carried out prior to the surfactant era have reported a decreasing need for assisted ventilation and oxygen supplementation after early indomethacin administration for closure of the PDA (Mahony et al. 1982, Kääpä et al. 1983).

4.2.2. Pneumothorax

In placebo-controlled studies indomethacin administration at <24 hours of age has had no effect on the incidence of pneumothorax in infants of birthweight <1000g (Hanigan et al. 1988) and <1750g (Rennie et al. 1986), although a reducing effect on a subgroup of infants with birthweight >999g has been suggested (Hanigan et al. 1988). In contrast, after treatment of symptomatic PDA, a trend has been seen toward a lower incidence of pneumothorax in infants of birthweight <1000g (Gersony et al. 1983).

4.2.3. Bronchopulmonary dysplasia

The incidence of BPD, diagnosed at 28 days of age, has not differed between premature infants of <1251g birthweight receiving prophylactic indomethacin treatment or placebo administration (Ment et al. 1994a) and in another study

indomethacin prophylaxis did not alter the incidence of BPD diagnosed at 36 weeks' postconceptional age in infants with < 28 weeks' gestation (Narayanan et al. 2000).

Indomethacin treatment for symptomatic PDA has had no effect on the duration of ventilation required (Merritt et al. 1979, Gersony et al. 1983) nor on the incidence of BPD at the age of four weeks in infants of <1750g compared to cases without such treatment (Gersony et al. 1983, Bada et al. 1989).

4.3. Cerebral effects

Indomethacin has been thought to exert its effects on cerebral haemodynamics at least partly via inhibition of PG synthesis (Leffler et al. 1985, Leffler and Busija 1987). However, the capacity of indomethacin to influence cerebral haemodynamics rapidly without change in prostanoid synthesis and evidence that other PG synthesis inhibitors such as ibuprofen lack the cerebral vasoconsrtictor effect in preterm infants would indicate that indomethacin-induced effects on cerebral blood flow are not wholly related solely to inhibition of PG synthesis (Van Bel et al. 1993b, Mosca et al. 1997, Patel et al. 2000). Direct effects of indomethacin on the smooth muscle cells by inhibition of calcium uptake and histamine release and elevating circulating endothelin levels have been surmised (Northover 1971, König et al. 1987, Therkelsen et al. 1994).

In newborn animals, indomethacin has been shown to reduce cerebral blood flow, to attenuate the cerebral hyperaemic response to hypoxia and hypercarbia and to improve the autoregulatory capacity of the cerebral vascular bed (Leffler et al. 1985, Van Bel et al. 1993a). Indomethacin also reduces the generation of oxygen free radicals during recovery from asphyxia, and pretreatment with the drug can reduce the ischaemia-induced alteration in the blood-brain barrier (Pourcyrous et al. 1993, Zuckerman et al. 1994). In newborn beagle pups indomethacin has been shown to promote germinal matrix microvessel maturation (Ment et al. 1992).

Human studies evaluating the effects of indomethacin on cerebral haemodynamics have usually been carried out in premature infants with

symptomatic PDA at postnatal ages up to one month. Both bolus or slower, >30 minutes' administration of indomethacin to premature infants has been associated with a drop in cerebral blood flow and volume as measured by Doppler ultrasonography or near-infrared spectroscopy (Mardoum et al. 1991, Austin et al. 1992, Patel et al. 2000). Continuous infusion for 36 hours, on the other hand, has had no effect on cerebral haemodynamics (Hammerman et al. 1995). A significant decrease in cerebral oxygen delivery after indomethacin administration has been reported in newborn infants and there is evidence of a reduction in the cerebral oxidized cytochrome oxidase concentration as a sign of decreased intracellular oxygenation after indomethacin infusion (McCormick et al. 1993, Liem et al. 1994, Mosca et al. 1997). The changes in cerebral haemodynamics have shown no correlation with the gestational age, birthweight or postnatal age of the infants (Mardoum et al. 1991, McCormick et al. 1993, Patel et al. 2000).

Yanowitz and coworkers (1998) found that prophylactic low-dose (0.1 mg/kg) indomethacin administration reduces the cerebral mean blood flow velocity and increases cerebral relative vascular resistance in premature infants of birthweight <1251 g and postnatal ages of 6 hours.

4.3.1. Intraventricular haemorrhage

The benefical effects of prophylactic treatment on IVH have been well established in infants weighing <1750g at birth (Fowlie 1996) and in placebo-controlled studies prophylactic indomethacin administration within the first 24 hours of life has significantly reduced the incidence of IVH (mainly grade II) in infants of birthweight <1301g (Bandstra et al. 1988) and < 1501g (Bada et al. 1989). Prophylactic indomethacin administration has also been associated with lower severity of IVH in infants of birthweight <1251g (Ment et al. 1994a) and there is no evidence that such treatment might cause an extension of IVH if administered to infants with grade I IVH (Ment et al. 1994b, Bada et al. 1989).

Gersony and coworkers (1983) reported an association between short-term indomethacin treatment of symptomatic PDA and a decreased incidence of IVH in 13 infants of birthweight <1000g if compared with 28 infants without indomethacin administration. However, the protective effect was not seen if the

whole study population of 421 infants of birthweight <1751g was considered (Gersony et al. 1983). Short indomethacin treatment has on the other hand been associated with an increased incidence and severity of IVH when compared with prolonged treatment (Rhodes et al. 1988).

4.3.2. Periventricular leukomalacia

In a study of 257 infants of gestational age <28 weeks receiving prophylactic or symptomatic treatment for DA, indomethacin administration prophylactically was held to lower the incidence of cystic periventricular leukomalacia (Narayanan et al. 2000). In contrast, no benefical effects of the drug were shown in a placebo-controlled trial of 61 infants <1251g birthweight (Ment et al. 1994b).

4.4. Renal effects

Ever since the first studies concerning indomethacin treatment for closure of PDA, renal dysfunction, including a reduction in urine output, a rise in blood urea nitrogen, increased serum creatinine and urinary osmolality and reduction in urine and serum sodium concentrations has been associated with indomethacin administration in premature infants (Friedman et al. 1976, Heymann et al. 1976, Seyberth et al. 1983b). Anuria, however, is a rarely described complication of the treatment (Barrington and Fox 1994). Closure of a PDA with indomethacin has also been shown to induce a significant, transient reduction in renal blood flow velocities, suppression of PG synthesis, a fall in plasma renin activity and a rise in plasma levels of arginine vasopressin in preterm infants (Seyberth et al. 1983b, Van Bel et al. 1991, Pezzati et al. 1999).

Indomethacin administration within the first 24 hours of life has been claimed to increase the incidence of oliguria in infants of birthweight <1301g (Bandstra et al. 1988), as well as a transient increase in plasma creatinine concentration, and a decrease in plasma sodium level and urine output has been observed after prophylactic indomethacin treatment in infants of birthweight <1501g (Bada et al. 1989).

Low urine output prior to indomethacin treatment has been held to predispose to symptomatic oliguria and the indomethacin dosage may also affect

the manifestation of renal failure (Ment et al. 1985, Bandstra et al. 1988, Rennie and Cooke 1991, Barrington and Fox 1994). However, renal dysfunction seems to be transient, normalization occurring within a few days (Friedman et al. 1976, Kääpä et al. 1983). Urinary output might also improve despite continued administration of the drug (Seyberth et al. 1983b, Bandstra et al. 1988).

4.5. Gastrointestinal effects

In newborn animals, postnatal indomethacin treatment has been shown to decrease the blood flow in the terminal ileum and block the autoregulation of intestinal oxygen consumption (Meyers et al. 1991). It may also increase the risk of bowel necrosis after temporary intestinal ischaemia (Krasna and Kim 1992). In premature infants intravenous indomethacin administration both prophylactically and for PDA closure has induced a significant reduction in superior mesenteric artery blood flow velocity (Coombs et al. 1990, Van Bel et al. 1990, Yanowitz et al. 1998), which reaches its nadir within 10 minutes after bolus administration, recovery occurring within a few hours. The reduction seems to be less severe and the time to maximum fall about half an hour longer after slow >30 minutes than after rapid infusion (Coombs et al. 1990).

The mechanism underlying vasoconstriction caused by indomethacin is still unknown, but an effect at least partly via inhibition of PG synthesis has been speculated (Konturek et al. 1982, Levine et al. 1988, Pezzati et al. 1999). Additionally, as the general protective effects, including inhibition of gastric acid secretion, stimulation of bicarbonate secretion and synthesis of mucus, as also an increase in the hydrophobicity of the gastric mucosa by increasing phospholipids are attributable to prostaglandins, the inhibition of prostaglandin synthesis with indomethacin further compromises intestinal defence mechanisms (Schoen and Vender 1989). Indomethacin-induced prostaglandin deficiency has also been held to weaken the resistance of the intestinal mucosa to microorganisms and/or their toxins (Robert and Asano 1977).

Sporadic cases of NEC or isolated intestinal perforations in the ileum or colon have been described both after indomethacin prophylaxis and after treatment

for PDA (Meyer et al. 1991, Rajadurai and Yu 1991, Ment et al. 1994a, Kumar and Yu 1997). Multiple gastric perforations after postnatal treatment for PDA have also been reported in preterm infants (Rajadurai and Yu 1991). Grosfeld and coworkers (1996) found an increased incidence of NEC and bowel perforation in infants after indomethacin administration for PDA compared to cases matched for gestational age and birthweight without PDA and indomethacin treatment. However, a meta-analysis showed only a trend toward an increasing incidence of NEC after postnatal prophylactic indomethacin treatment among infants weighing <1750g at birth (Fowlie 1996). Furthermore, GI complications, including NEC and isolated bowel perforation, have also been described without postnatal indomethacin exposure (Bada et al. 1989, Meyer et al. 1991).

Immaturity, birthweight <1000g and prolonged ventilator support seem to increase the risk of NEC and bowel perforation in indomethacin-treated infants (Rajadurai and Yu 1991, Grosfeld et al. 1996, Kumar and Yu 1997, Narayanan et al. 2000), but the duration of treatment has not had any effect on GI complications (Rhodes et al. 1988, Rennie and Cooke 1991).

4.6. Bleeding tendency

Postnatal indomethacin administration may cause platelet dysfunction, defined as absence of platelet aggregation and prolongation of bleeding time in preterm infants (Friedman et al. 1978, Corazza et al. 1984, Rennie et al. 1986) and normalization of the values after exposure can take more than a week (Friedman et al. 1978). Clinical signs of bleeding from the GI tract, transient occult haematuria and diffuse intravascular coagulopathy have been described in preterm infants after indomethacin administration (Friedman et al. 1978, Corazza et al. 1984, Peckham et al. 1984, Rennie et al. 1986). However, Ment and coworkers (1994a) found no significant difference in the incidences of excessive bleeding between infants <1251g with or without indomethacin exposure.

4.7. Other effects

4.7.1. Septicaemia

One report has suggested a significant increase in the incidence of septicaemia among 31infants of birthweight <1500g treated with indomethacin for PDA compared with 27 without exposure (Herson et al. 1988). Several other investigators report no such effect (Gersony et al. 1983, Mahony et al. 1985, Bandstra et al. 1988).

4.7.2. Mortality

Indomethacin treatment for symptomatic PDA has not been shown to influence mortality among infants born prematurely, although there are suggestions of hazardous effects of prolonged compared with short, one-day treatment (Gersony et al. 1983, Rennie et al. 1991, Grosfeld et al. 1996). On the other hand, a meta-analysis of prophylactic indomethacin suggested a trend toward a reduction in mortality rate in infants born <1750g (Fowlie 1996).

5. Adverse effects of combined use

There have been no studies of the effects of combined antenatal and postnatal indomethacin exposure on premature infants.

6. Long-term follow-up of patients with perinatal indomethacin exposure

6.1. Antenatal exposure

Two matched retrospective studies of 30 and 79 infants found no differences in neurodevelopmental outcome at 6 to 12 months (Al-Alaiyan et al. 1996) and 18 months (Souter et al. 1998) of age between children born prematurely with or without antenatal indomethacin exposure. In a prospective follow-up study by Salokorpi and coworkers (1996), 53 children with antenatal indomethacin

exposure had a poorer outcome (death or severe BPD and/or cerebral palsy and/or severe retinopathy of prematurity) at the corrected age of 12 months compared with 40 children with antenatal nylidrin exposure. Altogether 44 of these children underwent neurological examination at a corrected age of 18 months and the neurological development tended to be less favourable in the indomethacin than the nylidrin group. Growth of the children did not differ significantly between the groups (Salokorpi et al. 1996). Since, however, sample sizes in all of these follow-up studies have been small and follow-up rather short, it is very difficult to draw conclusions as to the long-term safety of maternal indomethacin use.

6.2. Postnatal exposure

A one-year follow up study of 52 infants revealed no differences in growth, incidence of vision or hearing problems, psychomotor and mental development or renal function between children after postnatal indomethacin therapy or surgical ligation of PDA (Merritt et al. 1979). Developmental tests at the age of 2-3 years also showed no differences between the groups (Merritt et al. 1982). Furthermore, equal growth, motor and cognitive development at one year of age was found in 24 children receiving postnatal indomethacin treatment for PDA and placebo (Yeh et al. 1981).

Recent follow-up studies have evaluated the long-term effects of postnatal indomethacin prophylactic treatment on children born prematurely. Prophylactic low-dose indomethacin treatment in infants of birthweights <1251 g seems not to affect cognitive outcome or incidence of cerebral palsy, deafness or blindness at 36 months' corrected age (Ment et al. 1996, Allan et al. 1997, Couser et al. 2000). At 54 months' corrected age, a similar incidence of cerebral palsy has been found in indomethacin- and placebo-treated infants, children treated with prophylactic indomethacin evincing even less mental retardation (intelligence quotient <70) and better language and social skills, and being less withdrawn than placebo-treated children (Ment et al. 2000).

AIMS OF THE STUDY

The purpose of the present study was to compare two postnatal indomethacin administration strategies and to evaluate the short- and long-term effects of perinatal indomethacin exposure in infants born at <33 weeks gestation.

The specific aims were:

1. to establish whether a prolonged low-dose course of indomethacin would produce a more complete closure rate and have fewer side-effects and better outcome compared with a short schedule in the management of haemodynamically significant PDA in preterm infants (I).

2. to identify the predictors of neonatal complications among preterm infants with antenatal, postnatal, both ante- and postnatal, or without any indomethacin exposure (II).

3. to establish whether perinatal indomethacin treatment has an influence on the frequency of oesophageal and gastric mucosal lesions and gastrointestinal symptoms in preterm infants (III).

4. to evaluate renal function, growth and macroscopic structure in early childhood and to investigate the possible independent effect of perinatal indomethacin exposure on abnormal renal findings in children born prematurely (IV).

SUBJECTS AND METHODS

1. Subjects and study design

The study comprised of one prospective randomized trial (I), two retrospective comparative trials (II, III) and one follow-up trial (IV). Altogether 332 subjects born at <33 weeks' gestation between the years 1991-1997 were included in the study; 241 of them participated in one, 78 in two and 13 in three trials. All subjects were treated at the neonatal intensive care units in Tampere (I-IV) or Oulu (I) University Hospitals.

1.1. Prospective trial (I)

Altogether 61 infants with a haemodynamically significant PDA with continuous left-to-right shunting were included in the study between the years 1993 and 1997. The contraindications for indomethacin treatment were: (1) presence of a heart defect dependent on a PDA, (2) pulmonary hypertension or a bidirectional shunt, (3) oliguria, (4) platelet count <60 x 10^9/L or a bleeding diathesis, (5) serum bilirubin >200 μmol/L, or (6) clinical or radiological evidence of NEC.

1.2. Retrospective trials (II, III)

Trial II involved all 240 infants born between the years 1991-1993. The trial III population comprised 69 infants born between the years, 1992-1997 who underwent upper GI tract endoscopy during the first four weeks of life and who had not received H_2-receptor antagonists, proton pump inhibitors or antacids before the endoscopy. The indications for the endoscopy were participation in a study where endoscopy was included in the protocol in 59 cases (trial I, Kuusela et al. 1997, Kuusela et al. 2000) and GI symptoms, including bleeding, feeding intolerance or failure to thrive, in 10 cases. The parents had given informed consent in all cases.

1.3. Follow-up trial (IV)

The original study population comprised 301 infants born between the years 1993 and 1996. Of these, 45 had died and altogether 85 were excluded because data on maternal indomethacin exposure were missing, the mother had received indomethacin less than 150 mg/day in cases without postnatal exposure, or ibuprofen had been used postnatally for closure of PDA. Eleven cases could not be contacted because of unknown address. The remaining 160 children were invited for examinations at ages of 2 to 4 years. The final study population consisted of 66 children whose parents consented to allow their children to participate in the study.

The birth characteristics of the infants studied and the type of indomethacin exposure involved are shown in Table 1. More detailed description of the study populations are presented in the original publications I to IV.

Table 1. Characteristics of the infants in trials I-IV

Study	Exposure	No of infants	Cumulative antenatal exposure (mg) median (range)	Cumulative postnatal exposure (mg) median (range)	Gestational age (wk) median (range)	Birthweight (g) median (range)
I	Postnatal short	31	0 (0-9100)	0.4 mg/kg	28 (24-32)	1128 (670-2030)
	Postnatal long	30	0 (0-2800)	0.7 mg/kg	27 (24-32)	1050 (580-2060)
II	Only antenatal	82	200 (25-5175)		30 (23-32)	1393 (550-2270)
	Only postnatal	37		0.57 (0.09-0.74)	29 (25-32)	1170 (430-2330)
	Combined ante- and postnatal	27	350 (25-5175)	0.58 (0.08-0.80)	27 (24-32)	1070 (595-2160)
	Controls	94			31 (23-32)	1435 (455-2470)
III	Ante- and/or postnatal	45	250 (50-9100)	0.41 (0.19-0.80)	28 (25-32)	1115 (690-1970)
	Controls	24			30 (25-32)	1303 (800-2330)
IV	Ante- and/or postnatal	31	500 (100-1645)	0.65 (0.37-1.20)	28 (24-32)	1150 (670-2060)
	Controls	35			31 (24-32)	1360 (680-2680)

2. Methods

2.1. Diagnostic criteria (I-IV)

Gestational age was estimated by obstetric dates and prenatal ultrasonography. Neonates with birthweights more than two standard deviations (SDs) under the norm for gestational age were considered small for gestational age (SGA). RDS was diagnosed if the infant yielded typical findings on chest X-ray films, needed oxygen supplementation for at least 24 hours, or had received surfactant. Diagnostic criteria for BPD were need of oxygen supplementation and typical findings on chest X-ray films at 36 weeks' postconceptional age (Shennan et al. 1988). IVH was classified according to Papile and colleagues (1978) and periventricular leukomalacia was defined as periventricular white matter cysts. The diagnosis of septicaemia required positive blood culture, an increased proportion of immature neutrophils (>20%) and elevated C reactive protein (>20). The diagnosis of NEC was made on modified Bell criteria (Walsh and Kliegman 1986) and severe NEC was diagnosed as pneumoperitoneum in abdominal radiography, bowel perforation at laparotomy or autopsy or bowel necrosis in postmortem examination. Oliguria was defined as urine output <1 mL/kg/h for more than 6 hours.

GI symptoms in trial III included bleeding (blood-stained gastric aspirates or blood in stools), tenderness of the stomach, vomiting or gastric feeding residuals severe enough to interrupt feeding for at least 24 hours. Visual endoscopic findings in the oesophagus and stomach were classified separately. The findings in the oesophagus were 1) intact mucosa, 2) mildly eroded, 3) moderate/strong erythema or erosion/ulcer, and in the stomach 1) intact mucosa, 2) mucosal friability and erythema, 3) gastropathy, 4) haemorrhage or erosion/ulcer. Histological results were classified as 1) normal, 2) inflammation, 3) haemorrhage or erosion/ulcer.

A diagnosis of a haemodynamically significant PDA was reached if the infant fulfilled the pertinent echocardiographic criteria and had at least three of the

following six clinical signs of cardiovascular dysfunction: (1) a systolic or continuous murmur at the left sternal edge, (2) an increased precordial impulse, (3) bounding peripheral pulses, (4) resting tachycardia, (5) unexplained deterioration of respiratory status, and (6) increased pulmonary vascular markings or cardiac enlargement or signs of pulmonary oedema on the chest radiograph. PDA closure was successful when an image of the PDA could not be obtained as patent and no shunt could be recorded on color flow Doppler imaging, or a pulsed Doppler search of the pulmonary end of the duct or a major ductal constriction with a nonsignificant residual flow was measured.

2.2. Drug treatment and follow-up in the prospective trial (I)

Infants randomized to the short course group received 3 doses of indomethacin intravenously, the initial dose administered being 0.2 mg/kg and following doses 0.1 mg/kg at 12-hour intervals. The long course group received 7 doses of 0.1 mg/kg at 24-hour intervals. All infants were weighed twice daily from the first dose of indomethacin and for 7 days thereafter. Urine output was monitored by weighing diapers.

2.3. Feeding practices in the neonatal intensive care unit

The practice in the unit was to withhold enteral feeding while administering inotropics. Otherwise enteral feeding was initiated on the first day of life if the infant was in stable condition. The infants were fed with their mother's milk and/or banked pooled breast milk via nasogastric tubes, using a bolus feeding technique with an initial dosage of 10-20 mL/kg/day, and maximum daily increments of 20 mL/kg. Parenteral nutrition was administered from the second day of life onwards until the infant reached full enteral feeds. Thereafter, infants of birthweights <1500 g received breast milk fortified with PreSemp® 5g/100mL milk up to 2000 g of weight. Formula was not used.

2.4. Laboratory measurements (I, IV)

In the prospective trial (I) serum sodium and potassium levels were measured twice a day, serum bilirubin and platelet levels once daily, and plasma creatinine and blood urea nitrogen levels at 2 days' intervals. In the follow-up study (IV) blood samples were drawn for determination of serum cystatin C, protein and plasma creatinine, sodium and potassium. Random spot urine samples were obtained for analysis for protein, calcium, creatinine and α_1-microglobulin content. Serum cystatin C concentrations were determined by a particle-enhanced turbidimetric immunoassay (Dako, Glostrup, Denmark) using a Hitachi 704 analyser (Ylinen et al. 1999). Plasma creatinine measurements (II, IV) were based on the Jaffe reaction (Bartels et al. 1972) by the same instrument. Urinary α_1-microglobulin was measured nephelometrically (Boehring BN II nephelometer, Dade Boehring, Marburg, Germany) with a sensitivity of about 5 mg/L.

2.5. Ultrasonographic measurements

Cranial ultrasonographic examination was performed through the anterior fontanelle with a 5 MHz scanner. The investigations were repeated at 1 to 3 days' intervals during the first week of life and at 1- to 2- week intervals thereafter until discharge.

In trial I, echocardiograms were taken in all infants with clinical signs of a PDA. Also in all ventilator-treated infants, echocardiography was used daily during the first 3 to 4 days of life and later in cases with an increased need of ventilatory support. Echocardiography was repeated in all patients on the third, ninth and fourteenth days after the first dose of indomethacin administered. Standard echocardiography with an Acuson 128/XP10 (Mountain View, Calif) scanner with a 7 MHz probe was used. Color and pulsed wave spectral Doppler scanning was used to define the direction and velocity of the ductal flow from parasternal and suprasternal views

Renal sonography examinations with an Acuson Sequoia (Mountain View, CA, U.S.A.) scanner were made to all the patients included in the follow-up study (IV). Both kidneys were scanned in prone, oblique and supine positions using 4V2

vector, 8C4 curvilinear and 8L5 linear transducers. Measurements of the kidney length were compared with a graph for length (Dinkel et al. 1985).

2.6. Gastroscopy (III)

Upper GI tract endoscopies were performed using a fiberoptic infant gastroscope (GIF-N30, Olympus Optical, Tokyo, Japan). The infants examined were in stable clinical condition and the need for premedication was considered individually. Endoscopy was performed under visual control, and blood pressure, heart rate and oxygen saturation were monitored throughout the procedure. Biopsy specimens were obtained from oesophagus and stomach, if possible. The contraindications for biopsy were thrombocytopenia or prolonged thromboplastin time. The biopsy specimens were formalin-fixed and embedded in paraffin wax.

2.7. Blood pressure (IV)

Blood pressure (BP) was measured by an oscillometric method (DINAMAP^{TM} Adult/Paediatric and Neonatal Vital Sings Monitor Model 1846 SX, Criticon, Inc., USA) on the right arm in sitting position, using a child cuff or a small adult cuff ensuring that it covered two thirds of the upper arm.

2.8. Glomerular filtration rate (IV)

The GFR was determined by the plasma clearance of ^{51}Cr-EDTA assessed by the single-injection method (Garnett et al. 1967). As a range of age standard GFR value 89 to 165 mL/min/1.73 m^2 was used (Goldsmith and Novello 1992).

2.9. Statistical methods

The data were analysed using the Statistic Package for Social Sciences (SPSS)/Win and the Graphpad Instat. Continuous data were analysed using independent samples t-test, if normally distributed, or Mann-Whitney U-test if not. Discrete data were analysed using the Chi-square test or Fisher's exact test. Differences between the mean values of variables at different times were assessed by analysis of variance for repeated measures (I). To clarify the effects of

indomethacin treatment on oesophageal and gastric mucosal findings, logistic regression analysis with enter method was used (III). To identify the factors affecting the outcome variables, logistic regression analyses with a backward stepwise method were performed (I-IV).

2.10. Ethics

The study designs of trials I-IV were approved by the ethics committees of the participating hospitals. In the prospective part of the study (I, part of III, IV) informed constent was obtained from the parents.

Special attention was focused on proper instrumentation, gentle handling of the infants and monitoring during the upper GI tract gastroscopy. Previous studies have proved that a gastroscopy examination is a safe bed-side procedure even in the most immature infants (Mäki et al. 1993, Ruuska et al. 1996).

RESULTS

1. Comparison of the effects of a short and prolonged course of indomethacin (I)

1.1. Closure of the patent ductus arteriosus

Clinically significant PDA was diagnosed at a mean (range) of 4.3 (4.4) days of age in the short course group and 3.1 (1.7) days of age in the long course group (p=0.71). Initial PDA closure occurred in 29 (94%) patients in the short course group and in 20 (67%) in the long (p=0.01). After the first treatment, a clinically significant reopening was diagnosed in 6 infants in the short course group and 2 infants in the long. After retreatment with indomethacin (six infants in both groups), the final closure rates with indomethacin in the respective groups were 28 (90%) vs 19 (63%), p=0.02 and the PDA was surgically ligated in 2 (7%) vs 9 (30%) patients, p=0.02. In infants of gestational ages <26 weeks, PDA closure with indomethacin was successful in only three of the five patients who received the short and none of the five infants receiving the long course.

In the logistic regression analysis, a long course of indomethacin (odds ratio (OR) 11.06, 95% confidence interval (CI) 1.42 to 86.29) correlated with indomethacin treatment failures. In an analysis of 49 cases with primary response to indomethacin, low gestational age (OR 1.52, 95% CI 0.90 to 2.55) seemed to involve a trend toward increased risk of ductus reopening.

1.2. Outcome and side-effects

There were no significant differences between the groups in the incidence of grade I-II and III-IV IVH, periventricular leukomalacia and BPD. Also mortality rates and the duration of assisted ventilation were similar. The median duration of

oxygen supplementation was shorter in the short course group, 27 (range 2.5-450) days than in the long, 54 (range 5-180) days (p=0.04).

Side-effects of the short and long courses of indomethacin are shown in Table 2. Analysis of variance for repeated measures revealed significantly lower mean (SD) blood urea nitrogen levels in the short compared to the long course group (5.7 (2.5) mmol/L vs 7.4 (3.2) mmol/L, p=0.03). Urea retention >3.6 mmol/L was more frequent in the long course group. All renal side-effects were transient. Symptoms of NEC were more common in the long course group, but the numbers of grade III cases, 3 (10%) in the short course group and 5 (17%) in the long, did not differ (p=0.47). Likewise the numbers of patients suffering at least one severe adverse event, including significant bleeding tendency, NEC with intestinal perforation and symptomatic oliguria, were similar in the groups. All of these events occurred in infants of gestational ages <28 weeks.

Table 2. Side-effects of the short and long course of indomethacin

Variable	Short course (n=31)	Long course (n=30)	P value
Urine output <1 mL/kg/h	13	3	0.35
Serum sodium <130 mmol/L	58	40	0.20
Serum creatinine >150 µmol/L	23	10	0.30
Blood urea nitrogen >3.6 mmol/L	10	43	0.004
Serum bilirubin >200 µmol/L	10	7	1.00
Platelet levels <60 x 10^9/L	13	7	0.67
Bleeding diathesis	19	13	0.73
Gastrointestinal haemorrhage	26	20	0.76
Necrotizing enterocolitis	26	53	0.04
Severe adverse event*	13	20	0.51

Data are shown as percentages

* significant bleeding tendency, NEC with intestinal perforation or symptomatic oliguria

In the logistic regression analysis, a long course of indomethacin (OR 3.28, 95% CI 1.04 to 10.36) and treatment with inotropics (OR 7.49, 95% CI 1.47 to 38.19) had a significant association with the development of NEC. In contrast, low

gestational age (OR 0.50, 95% CI 0.28 to 0.88) was the significant risk factor associated with severe adverse events.

2. Neonatal complications after perinatal indomethacin treatment (II)

The infants with combined ante- and postnatal exposure suffered most neonatal complications (Table 3). Four infants, one in the antenatal exposure group and three in the combined exposure group, had pneumoperitoneum and an isolated bowel perforation without necrosis. The incidence of symptomatic PDA was 27 out of 109 (25%) among infants with antenatal indomethacin exposure and 37 out of 131 (28%) in infants of no maternal indomethacin treatment.

Table 3. Outcome variables in trial II

Variable	No exposure group (n=94)	Antenatal exposure group (n=82)		Postnatal exposure group (n=37)		Combined ante- and postnatal exposure group (n=27)	
IVH							
- grade I-II	6	15	0.02	8	0.69	15	0.09
- grade III-IV	9	9	0.79	22	0.07	26	0.02
Severe NEC	2	5	0.42	8	0.14	26	<0.001
Septicaemia	5	5	1.00	14	0.14	30	0.001
Pneumothorax	11	7	0.60	22	0.16	30	0.03
RDS	24	27	0.85	54	0.002	52	0.01
BPD	2	4	1.00	19	0.002	26	<0.001
Mortality	21	16	0.44	11	0.21	22	1.00

Data are shown as percentages followed by p values. Comparison with the No exposure group.
IVH=intraventricular haemorrhage, NEC=necrotizing enterocolitis, RDS=respiratory distress syndrome, BPD=bronchopulmonary dysplasia

In the logistic regression analysis, low gestational age (OR 0.72, 95% CI 0.61 to 0.86) emerged as the significant risk factor for grade I-II IVH. SGA correlated with a decreased (OR 0.14, 95% CI 0.02 to 0.88) and RDS (OR 4.57, 95% CI 1.75 to 11.91) and low birthweight (OR 0.997, 95% CI 0.995 to 0.996) with an increased incidence of grade III-IV IVH. Severe NEC correlated with RDS (OR 3.24, 95% CI 1.00 to 10.49), low birthweight (OR 0.998, 95% CI 0.997 to 0.999) and combined ante- and postnatal indomethacin administration (Table 4). Low birthweight (OR 0.998 95% CI 0.996 to 0.999) and combined ante- and postnatal exposure (Table 4) were also significant risk factors for septicaemia.

Table 4. Indomethacin covariates associated with IVH grade I-II, severe NEC and septicaemia

Variable	IVH grade I-II	Severe NEC	Septicaemia
Only antenatal exposure	3.22; 0.81 to 12.78	2.18; 0.38 to 12.58	1.10; 0.28 to 4.39
Only postnatal exposure	0.42; 0.05 to 3.52	2.01; 0.30 to 13.46	2.20; 0.57 to 8.45
Combined ante- and postnatal exposure	0.54; 0.06 to 4.88	8.65; 1.56 to 47.86	9.32; 2.36 to 36.92
Other antenatal indomethacin covariates:			
- dose ≥150 mg/day	4.49; 1.40 to 14.38		
- cumulative dose ≥150 mg	3.38; 1.29 to 8.87	4.24; 1.25 to 14.39	
- duration >2 days	3.89; 1.41 to 10.71	4.36; 1.10 to 17.36	

Data are shown as OR; 95% CI

In the further analysis of antenatal indomethacin administration, doses of antenatal indomethacin ≥150 mg/day, duration of treatment >2 days and cumulative administration ≥150 mg were significant risk factors for grade I-II IVH. Duration of antenatal treatment >2 days and cumulative dose ≥150 mg correlated with an increased risk of severe NEC (Table 4). Indomethacin had no association with BPD, pneumothorax or RDS and the interval between last dose of maternal indomethacin and delivery showed no significant correlation with neonatal morbidity.

3. Effects of perinatal indomethacin exposure on the gastrointestinal tract (III)

3.1. Endoscopic findings in the upper gastrointestinal tract

All the infants underwent macroscopic examination of oesophagus and stomach. Microscopic examination of oesophagus was performed to 18 (40%) infants in the study group and 22 (92%) infants in controls and microscopic examination of

stomach to 40 (89%) and 21 (88%) infants, respectively. Macroscopic and microscopic findings in the upper GI tract were similar in the two groups (Figure 2).

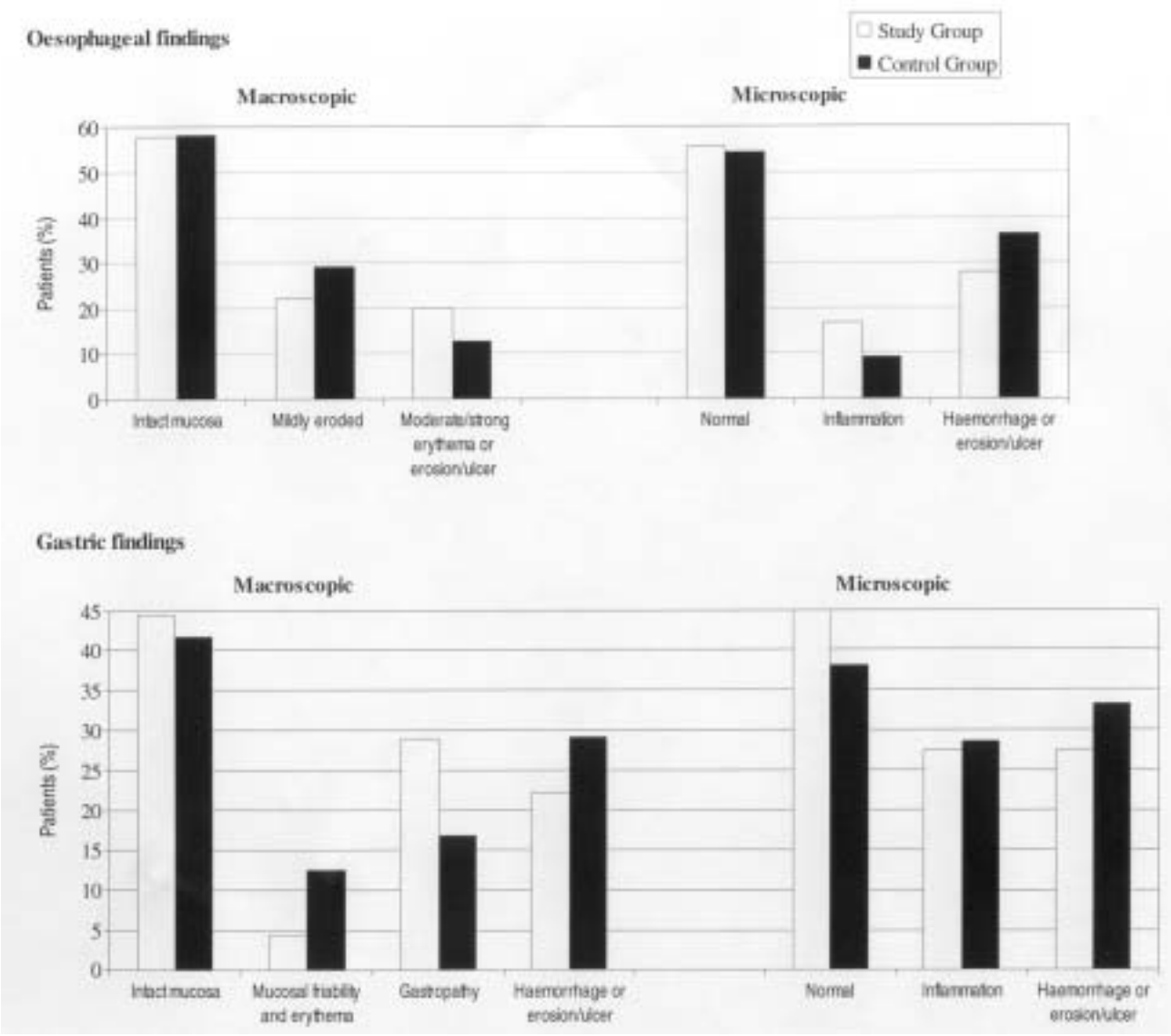

Figure 2. Mucosal findings in oesophagus and stomach

The median (range) interval between the last antenatal and perinatal indomethacin dose and endoscopy was shorter in cases with abnormal than in those with normal oesophageal findings, as was the corresponding interval between the last perinatal indomethacin dose and endoscopy in cases with

abnormal and normal gastric findings, respectively (Figure 3). In the logistic regression analysis, the interval between endoscopy and the last antenatal (OR 0.85; 95% CI 0.73 to 0.99) and perinatal (OR 0.72; 95% CI 0.56 to 0.94) indomethacin dose showed an association with abnormal oesophageal findings, the abnormal gastric findings being associated with a short interval between endoscopy and the last perinatal indomethacin dose (OR 0.81; 95% CI 0.67 to 0.97).

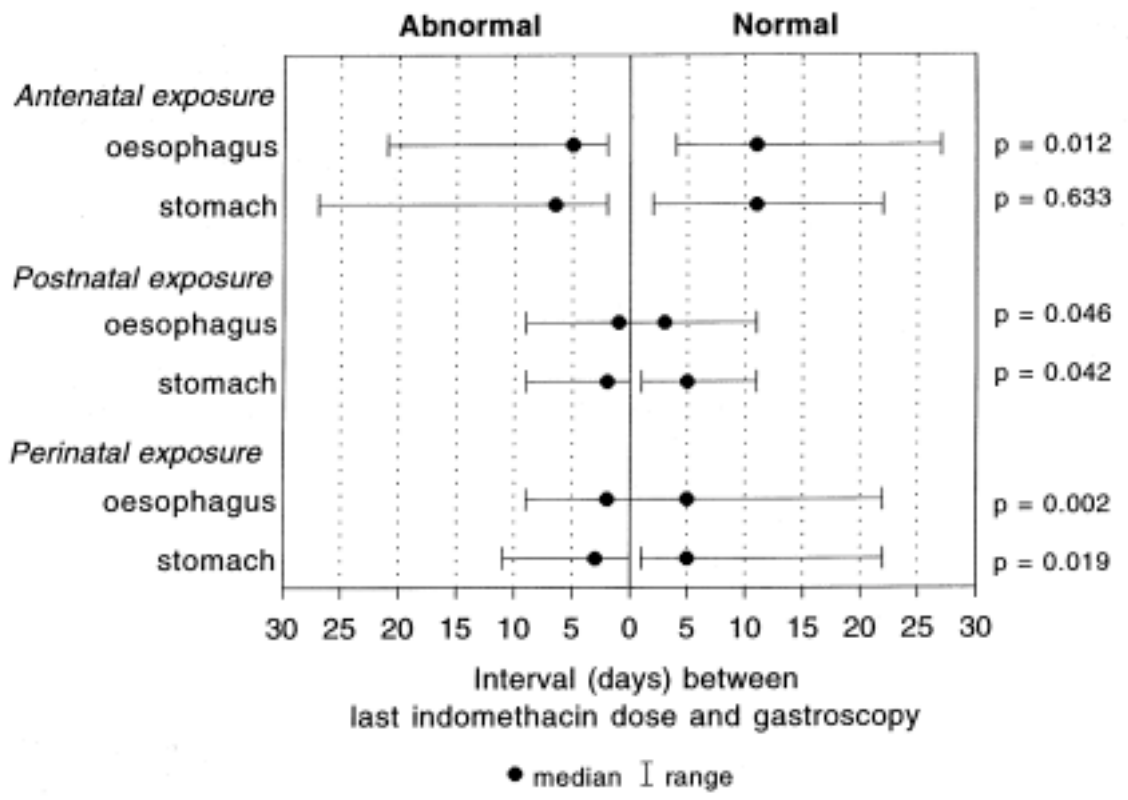

Figure 3. Median (range) interval between indomethacin dose received and endoscopy

In the multiple logistic regression model, short duration of enteral nutrition (OR 0.80, 95% CI 0.69 to 0.92) was the risk factor associated with abnormal oesophageal findings and gestational age (OR 1.39, 95% CI 1.02 to 1.91) and presence of ventilator treatment at the time of endoscopy (OR 3.53, 95% CI 1.05 to 11.87) with abnormal gastric findings.

3.2. Gastrointestinal symptoms

GI symptoms prior to endoscopy are presented in Table 5. In addition, 19 out of 42 (45%) infants with postnatal indomethacin exposure had GI symptoms during treatment. Five infants, all of them exposed to perinatal indomethacin, developed grade II-III NEC.

In the logistic regression analysis, perinatal indomethacin treatment (OR 6.38, 95% CI 1.23 to 33.10) was the only significant risk factor for GI symptoms.

Table 5. Gastrointestinal symptoms before endoscopy

Variable	**Infants without perinatal indomethacin exposure (n=24)**	**Infants with perinatal indomethacin exposure (n=45)**	**p value**
Bleeding	4	7	
Tenderness of the stomach	0	7	
Vomiting/ Gastric feeding residuals	4	16	
Total	8	29	0.07

Data are shown as percentages.

4. Renal effects of perinatal indomethacin exposure in early childhood (IV)

4.1. Renal function tests

Plasma creatinine, sodium and potassium, and serum protein levels did not differ significantly between the infants with and without perinatal indomethacin exposure. Mean (SD) serum cystatin C concentrations were slightly higher in the controls than in the study group (1.02 (0.17) mg/L vs 0.92 (0.17) mg/L, p=0.03). However, cystatin C values were lower than 1.4 mg/L in all children studied. Urine calcium/creatinine and protein/creatinine ratios were similar between the groups and none had significant tubular proteinuria measured as urine α_1-microglobulin concentrations. One child in each group had GFR 86 mL/min/1.73 m^2.

Systolic and diastolic BPs did not differ between the groups. Three children with perinatal indomethacin exposure and two without had both systolic and diastolic BPs higher than the 95th percentile for age (Horan et al. 1987).

4.2. Renal ultrasonographic findings

Kidney sizes for height were normal in both groups. Three children, two in the study group and one of the controls had size disproportion, i.e. a size difference of more than one SD between the kidneys.

Renal structural abnormalities were found in 10 (14%) of the children studied (Table 6). In the logistic regression analysis, duration of furosemide treatment (OR 1.18, 95% CI 1.03 to 1.35) and duration of umbilical artery catheter (UAC) use (OR 1.26 95% CI 1.00 to 1.58) had an independent association with these abnormalities.

Table 6. Clinical characteristics and findings in renal ultrasonographic examination in children with abnormal structural findings

Patient	Gestational age (wk)	Perinatal indomethacin treatment	Furosemide treatment (days)	Umbilical artery catheter use (days)	Renal ultrasonographic findings
1	29	+	20	9	Nephrocalcinosis
2	30	+	17	16	Enlarged kidneys (+2 SD) Decreased corticomedullary differentiation Unilateral prehydronephrosis
3	28	+	48	0	Size disproportion
4	28	+	14	18	Unilateral nephrectomy Nephrocalcinosis
5	26	+	11	8	Size disproportion
6	32	-	0	0	Nephrocalcinosis
7	32	-	0	0	Unilateral hydronephrosis
8	30	-	11	12	Size disproportion
9	32	-	0	0	Unilateral hydronephrosis
10	26	-	1	3	Decreased corticomedullary differentiation

DISCUSSION

1. Methodological aspects (I-IV)

The present series evaluated the effects of perinatal indomethacin exposure on newborn infants born at <33 weeks' gestation, compared two postnatal administration regimens for PDA and evaluated renal findings in early childhood in children with and without perinatal indomethacin exposure.

In the prospective randomized trial the groups were well matched with respect to antenatal and clinical characteristics. Allocation to either postnatal indomethacin treatment schedule was determined using sealed envelopes. It was thus not possible to predict which group a newly admitted infant would enter. Echocardiograms were performed by investigators who were unaware of the group assignment of the infants (I).

Heterogeneity of the groups was a problem in the retrospective trial (II), the infants with combined ante- and postnatal indomethacin exposure being the most immature, smallest, sickest and at highest risk of adverse events. Thus, much attention was paid to the logistic regression analysis.

Also in the follow-up trial (IV) and in trial III the infants with perinatal indomethacin exposure were sicker and more premature than those not exposed and it was not possible to find well-matched control cases according to their neonatal characteristics. Indomethacin was usually used antenatally for prevention of preterm labor as the last alternative to tocolysis. Furthermore, infants with symptomatic PDA tended to be more premature than those without. Thus, the probability of indomethacin exposure seemed to increase with decreasing gestational age.

The percentage of refusal among the children invited to the follow-up trial (IV) was 59%. The main reasons for refusal included living at a distance from the

hospital, the child's fearfulness, and the complexity of the study protocol. Assessment without GFR study was then suggested and in six cases the parents allowed their children to go through this more simple study protocol. Analysis of the perinatal characteristics revealed no differences between refusals and cases examined. It may thus be suggested that the risk of selection bias is small and the results representative of the whole original study population.

2. Comparison of short and long courses of postnatal indomethacin treatment

2.1. Closure of the ductus arteriosus

Prolonged low-dose indomethacin administration for symptomatic PDA would appear to be associated with lower initial closure rates and a higher need for surgical ligation compared with the short three-dose course in infants <33 weeks' gestation. The reopening rate did not differ between the groups. Contrary to our results, previous trials have suggested higher initial response rates and lower reopening rates after six days' indomethacin therapy for PDA compared with two- or three-dose regimens (Rhodes et al. 1988, Rennie and Cooke 1991). It is difficult, however, to compare their results with ours because of differences in administration routes, ages of the infants at commencement of treatment and the methods of PDA diagnosis. Poor ductus constriction has been associated with low plasma indomethacin levels, although controversial opinions have also been presented (Brash et al. 1981, Ment et al. 1988). In this study the initial dose in the short course group was 0.2 mg/kg, whereas in the long course group it was 0.1 mg/kg. Thus, it may be possible that the lower initial dosage of indomethacin could not elevate the plasma drug level sufficiently and therefore constriction failed in the long course group.

2.2. Side-effects

Previous studies have pointed to low urine output before indomethacin treatment as a risk factor underlying drug-induced oliguria in preterm infants (Barrington

and Fox 1994). In the present study the fact that indomethacin treatment was initiated at the earliest on the second day of life, at the time when diuresis has usually already started (Lorenz et al. 1995), may have protected the infants against symptomatic oliguria. It is also possible that indomethacin dosage affects the urinary output (Ment et al. 1985) and the relatively low dosages used here might be related to the low rates of oliguric cases in both groups. Furthermore, elevation in blood urea nitrogen levels was more common in the long course group, on contrast to the findings of Rennie and Cooke (1991). However, renal side-effects were transient, as also previously reported (Friedman et al. 1976, Seyberth et al. 1983b).

The incidence of NEC (39%) and bowel perforation (13%) was similar to that previously reported in preterm infants treated with indomethacin for symptomatic PDA (Grosfeld et al. 1996). A lower dose of indomethacin does not seem to protect against NEC. On the other hand, a long course of indomethacin was associated with an increased incidence of NEC, a phenomenon not found in a previous comparative study (Rhodes et al. 1988)

2.3. Infants <28 weeks' gestation

Poor initial response and high DA reopening rate after postnatal indomethacin therapy, as also an increased need of surgical ligation of PDA in most premature infants have previously been reported (Rajadurai and Yu 1991, Trus et al. 1993, Weiss et al. 1995). The explanation can be the increased reactivity of the DA after initial constriction, and decreased ability to develop ductal vessel wall hypoxaemia in most immature infants, this preventing anatomic closure (Clyman et al. 1985, Clyman et al. 1999b). Furthermore, major complications, especially serious GI pathology, have been reported to occur mainly in most premature infants connected with indomethacin treatment for PDA (Rajadurai and Yu 1991, Trus et al. 1993, Kumar and Yu 1997). Here likewise all infants with severe adverse effects were born at <28 weeks' gestation. The question whether surgical ligation should be considered the optimal primary treatment for PDA in the most premature infants needs further investigation.

3. Gastrointestinal effects and perinatal indomethacin exposure

3.1. Necrotizing enterocolitis and isolated bowel perforations

Indomethacin tocolysis has been connected with postnatal GI pathology, including grade II-III NEC and GI bleeding (Vanhaesebrouck et al. 1988, Norton et al. 1993) and a duration of antenatal exposure >2 days and exposure within one day before delivery have been seen to be a risk factor for NEC (Major et al. 1994). After birth, both PDA and indomethacin treatment have been shown to disturb mesenteric blood flow in premature infants (Coombs et al. 1990), which might lead to mucosal hypoxia, injury and an increased risk of NEC. An association has also been reported between postnatal indomethacin administration for PDA and GI complications, including NEC and haemorrhage (Friedman et al. 1978, Grosfeld et al. 1996). In the present study, perinatal indomethacin administration seemed to be a risk factor for GI symptoms, including bleeding and feeding intolerance. Indomethacin exposure both ante- and postnatally, prolonged antenatal indomethacin exposure of more than two days and from already a quite moderate cumulative antenatal dose of 150 mg onwards appear to be associated with severe, grade III NEC. Our finding that the interval between the last dose of maternal indomethacin and delivery and only postnatal indomethacin exposure did not correlate with the occurrence of NEC in infants born at <33 weeks' gestation, does not support the results of previous works (Major et al. 1994, Grosfeld et al. 1996). Isolated bowel perforations, previously associated with both antenatal and postnatal indomethacin exposure (Norton et al. 1993, Grosfeld et al. 1996), were seen in 25% of our NEC cases, which further confirms the conception that such perforations represent pathognomonic complications related to indomethacin exposure in preterm patients.

3.2. Mucosal findings in the upper gastrointestinal tract

Gastro-oesophageal endoscopic findings, including upper GI tract inflammation, haemorrhage, erosions and ulcers have been reported in both adults and children

after oral indomethacin treatment (Lanza et al. 1979, Mulberg et al. 1993). In the present study a short interval between the last dose of perinatal indomethacin administered and endoscopy seems to be a risk factor for upper GI tract lesions, but a direct association between perinatal indomethacin and abnormal endoscopic findings could not be shown. Here both the antenatal and postnatal medication were administered via the parenteral route. An explanation might also be the variable interval between drug administration and endoscopy. It is not known how long the indomethacin effect on the intestinal mucosa may persist in preterm neonates, but in an adult study, gastroduodenal damage caused by indomethacin has been shown to resolve within one week despite continuous oral indomethacin treatment (Shorrock and Rees 1992).

4. Other risk factors affecting the mucosa of the upper gastrointestinal tract

4.1. Nutrition

In the present study, increased duration of enteral feeding seemed to protect the oesophageal mucosa in premature infants. Breast milk, used for all infants studied, contains surface-active phospholipids (Hamosh et al. 1999) and it improves gastric emptying in comparison with formula milk (Ewer et al. 1994). The beneficial effects of enteral nutrition might also be explained by the facts that it elevates gastric pH (Ephgrave et al. 1990), potentiates maturation of oesophageal peristalsis (Gryboski 1965) and may increase mesenteric blood flow velocity in preterm infants (Maruyama et al. 1999).

4.2. Gestational age

Increasing gestational age seemed to correlate with an increased risk of gastric mucosal lesions, a finding not previously reported. Premature infants have lower gastric acid secretion than full-term infants (Marino et al. 1984) and sick preterm patients have a lower gastric pH than those with stable condition (Sankaran et al. 1984), which may partly explain the result. In addition, the change in gastric

parietal cell location during the third trimester (Kelly et al. 1993) may also affect the mucosal response to gastric acid with increasing maturation of the infant.

4.3. Ventilator treatment

A high prevalence of oesophageal and gastric lesions, including inflammation, haemorrhage and ulcerations, has been described in critically ill infants (Adeyemi et al. 1979, Mäki et al. 1993) and a lack of correlation between endoscopic findings and GI symptoms has also been shown (Mäki et al. 1993). The need for ventilator assistance is an indicator of the severity of illness and stress in a premature infant and ventilator treatment also entails stress by reason of its invasive nature. The present association of an increased risk of gastric mucosal lesions among the ventilator-treated preterm infants supports previous data on stress-induced gastric lesions.

5. Intraventricular haemorrhage and perinatal indomethacin administration

The present findings suggest an increased risk of grade I-II, but not grade III-IV IVH in infants with daily and cumulative antenatal indomethacin exposure ≥150 mg and duration of antenatal treatment over two days, without any dependence on the interval between the last dose of maternal indomethacin and delivery. In previous reports, the association of indomethacin tocolysis with the risk of IVH in preterm infants has been controversial. Supporting the present results Norton and associates (1993) report an increased incidence of grade II IVH after antenatal exposure to indomethacin in infants born at <31 weeks' gestation. There are also reports of hazardous effects of maternal indomethacin treatment within 48 hours of delivery, by increasing grade I-II and III-IV IVHs in premature infants (Souter et al. 1998). In contrast, several trials have not shown such association, even in preterm cases born within two days of antenatal exposure (Gardner et al. 1996, Vermillion and Newman 1999, Panter et al. 1999).

Postnatal indomethacin administration during the first 24 hours of life has been shown to lower the incidence of IVH in premature infants (Fowlie 1996).

Consistently with the findings of Gersony and coworkers (1983), postnatal indomethacin administration here after the first 24 hours of life for the treatment of PDA did not affect the incidence of IVH. Indomethacin exposure both ante- and postnatally seemed neither to protect from nor to increase the risk of IVH.

6. Septicaemia and perinatal indomethacin administration

Antenatal indomethacin administration has not been found to affect the incidence of septicaemia in premature infants (Norton et al. 1993, Major et al. 1994). Also several investigators report no association between postnatal indomethacin administration and septicaemia (Gersony et al. 1983, Mahony et al. 1985, Bandstra et al. 1988). On the other hand, an increased risk of this condition was found among the present preterm infants with combined ante- and postnatal indomethacin exposure. The finding is partly in accordance with a report by Herson and coworkers (1988), suggesting such risk in very low birthweight infants receiving postnatal indomethacin treatment for PDA. The mechanism behind this is unclear. At least no effect of indomethacin on the function of polymorphonuclear leukocytes has been found (Herson et al. 1988).

7. Pulmonary complications and perinatal indomethacin administration

Previous work has suggested an increased risk of pulmonary complications, including RDS and BPD after indomethacin tocolysis (Eronen et al. 1994, Van Overmeire et al. 1998). This cannot be confirmed by the present data. Thus, the issue remains controversial and calls for further investigation.

8. Long-term renal findings

8.1. Effects of perinatal indomethacin exposure on renal function

In the present study, no long-term effects of perinatal indomethacin exposure on renal function were found in children born at <33 weeks' gestation, supporting earlier findings of normal serum creatinine and blood urea nitrogen concentrations, and urinanalysis during and after postnatal indomethacin administration in children born prematurely and examined at the age of one year (Merritt et al. 1979, Peckham et al. 1984). On the other hand, in children born preterm with a history of renal calcification as neonates, evidence of renal dysfunction has been found upon examination at the ages of 1-2 years (Downing et al. 1992) and 4.5 years (Ezzedeen et al. 1988). There are also suggestions of abnormal renal function in children born preterm without such a history, studied at the age of 4-5 years (Jones et al. 1997). In our follow-up study, almost all cases, with or without exposure to indomethacin, had good renal function, only two children having GFR values marginally lower than normal. Neither of these had nephrocalcinosis at ultrasonographic examination.

8.2. Factors affecting renal macroscopic structure

8.2.1. Perinatal indomethacin exposure

Long-term effects of perinatal indomethacin exposure on renal structure have not previously been studied. In the present series indomethacin administration perinatally had no effects on renal structure in children born at <33 weeks' gestation and studied at the age of 2-4 years. This lends further support to the assumption that the renal effects of indomethacin are transient.

8.2.2. Umbilical artery catheter use

Duration of UAC insertion in the neonatal period seemed here to be a risk factor for renal structural abnormalities. In a follow-up trial by Seibert and coworkers

(1991) structural abnormalities, including renal size below normal and size discrepancy between the kidneys, were found at the age of 3-3.5 years in children with a history of UAC thrombosis in their neonatal period. In our patients, management practices for UAC use, including placement above the renal arteries and catheter use as a calcium infusion route, may have increased the risk of neonatal UAC thrombosis (Seibert et al. 1987). Only one case in our series had had symptoms of UAC thrombosis during the neonatal period, but UAC can cause abnormalities in renal haemodynamics also in the absence of clinical symptoms (Glickstein et al. 1994).

8.2.3. Furosemide treatment

Furosemide treatment in the neonatal period is a well-known risk factor associated with renal structural abnormalities, i.e. renal calcification in preterm infants (Jacinto et al. 1988, Pope et al. 1996). The duration of neonatal furosemide administration seemed to be associated with the risk of structural abnormalities in the present study, two of the cases evincing renal calcifications present. Nephrocalcinosis apparently has a tendency to resolve with increasing age (Pope et al. 1996, Saarela et al. 1999) and this might already have occurred in some of our furosemide-exposed cases. The mechanism whereby furosemide might increase the risk of abnormalities other than nephrocalcinosis is unclear.

CONCLUSIONS

1. A 7 days' administration regimen of low-dose indomethacin to preterm infants with symptomatic PDA is associated with less effective primary closure, a higher rate of surgical ligations and an increased risk of adverse effects if compared with the standard 3-dose course. Thus, a prolonged course offers no advantage compared to a short course.

2. Indomethacin exposure both ante- and postnatally increases the incidence of NEC with bowel perforation and septicaemia, and antenatal indomethacin administration correlates with an increased incidence of grade I-II IVH in infants born at <33 weeks' gestation.

3. Recent or ongoing perinatal indomethacin treatment correlates with an increased risk of oesophageal and gastric mucosal lesions and increases the risk of gastrointestinal symptoms in neonates born at <33 weeks' gestation.

4. Perinatal indomethacin treatment would not appear to affect renal growth, macroscopic structure or function in early childhood in children born at less than 33 weeks' gestation.

5. Later renal macroscopic structural abnormalities correlate with the duration of umbilical arterial catheter use and duration of furosemide administration during the neonatal period in children born at <33 weeks' gestation. Renal follow-up can be recommended in such cases.

SUMMARY

Indomethacin, a prostaglandin synthetase inhibitor, is used antenatally as a tocolytic agent and in the treatment of polyhydramnios, and postnatally for pharmacological closure of PDA. Recently, postnatal indomethacin administration during the first 24 hours of life has been shown to reduce the incidence of IVH in infants born prematurely. However, both antenatal and postnatal indomethacin exposure have been associated with significant adverse effects, including GI pathology, bleeding tendency and renal dysfunction during the neonatal period in preterm patients. Few data are available regarding the long-term effects of perinatal indomethacin use on renal macroscopic structure and function in such patients.

The study objective was to establish whether a prolonged low-dose course of indomethacin for symptomatic PDA would produce a more complete closure rate, have fewer side-effects and correlate with a better outcome compared with a short schedule, and to identify the association of perinatal indomethacin exposure with neonatal complications and oesophageal and gastric mucosal lesions in newborn infants of gestational ages <33 weeks. In addition, renal function, growth and structure in early childhood were evaluated, and the possible independent effect of perinatal indomethacin exposure on abnormal renal findings was investigated in children born at <33 weeks' gestation.

In the randomized study, initial PDA closure occurred more often (94%) in the short three-dose (0.2 mg-0.1mg-0.1mg/kg at 12 hours' intervals) group (N=31) than in the group (N=30) treated on the long, 7-day schedule (0.1 mg/kg at 24 hours' intervals) (67%), but the sustained closure rates after the first course were not different (74% vs 60%). The final closure rate after repeated indomethacin administration was higher (90%) in the short course group compared with the long (63%). Surgical PDA ligations were less frequent in the short course group (7% vs 30%). This group also had a shorter duration of oxygen supplementation, less

frequent symptoms of NEC and less marked tendency to urea retention. Other neonatal morbidity and mortality rates were similar in the groups.

Among 240 cases born at less than 33 weeks' gestation studied retrospectively, antenatal indomethacin treatment for longer than two days and a daily or cumulative dosage ≥150 mg correlated with an increased risk of grade I-II IVH. Combined ante- and postnatal indomethacin exposure, cumulative antenatal exposure ≥150 mg and duration of maternal treatment over two days were associated with an increased risk of NEC and combined indomethacin exposure with an increased risk of septicaemia. There was no independent association between indomethacin exposure and pneumothorax, BPD or RDS.

Abnormal findings were equally common in 69 infants with (N=45) and without (N=24) perinatal indomethacin exposure who underwent upper GI tract endoscopy during their neonatal period. A short interval between the last perinatal indomethacin dose and endoscopy correlated with oesophageal and gastric mucosal lesions. Perinatal indomethacin administration also increased the risk of GI symptoms in retrospective analysis of the cases. In addition, a short duration of enteral feeding before gastroscopy was a risk factor associated with oesophageal mucosal lesions. The risk of abnormal gastric mucosal findings was increased in conjunction with ventilator treatment at the time of endoscopy and with increasing gestational age of the patient.

Renal growth and structure, studied by ultrasonography, and function, evaluated by ^{51}Cr-EDTA, blood tests, urineanalysis and blood pressure measurements, were good in 66 preterm-born children with (N=31) and without (N=24) perinatal indomethacin exposure, at the age of 2-4 years. The mean serum cystatin C concentrations were slightly higher in the group without perinatal indomethacin administration, but in all cases the values were within normal limits. Concentrations of plasma creatinine, sodium and potassium, and serum protein were similar between the groups, as were also urine protein/creatinine and calcium/creatinine ratios. None had tubular proteinuria. Abnormal GFR (<89 ml/min/1.73 m^2) was found in only one child in each group. Renal structural abnormalities were found in altogether 10 (14%) of the children, five cases in each

group. The duration of UAC use and furosemide treatment emerged as risk factors for renal structural abnormalities.

In conclusion, a prolonged low-dose indomethacin regimen offers no advantage compared with a short course in the management of a symptomatic PDA in preterm infants. Preterm infants exposed to antenatal indomethacin might run an increased risk of grade I-II IVH and those with both ante- and postnatal exposure an increased risk of NEC and septicaemia. The risk of adverse effects on the infants seems to increase with increasing duration of both antenatal and postnatal indomethacin uses. Recent perinatal or current postnatal indomethacin treatment may also increase the risk of GI symptoms and oesophageal and gastric mucosal lesions in preterm infants. In addition, short duration of enteral feeding is associated with an increased risk of oesophageal, and increasing gestational age and ventilator treatment with gastric mucosal lesions. Perinatal indomethacin exposure would not appear to affect long-term renal growth, macroscopic structure or function in children born prematurely, but duration of UAC use and furosemide treatment seem to be risk factors underlying subsequent renal macroscopic structural abnormalities.

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