Acta Pædiatr 86: 289–93. 1997

Pharmacokinetics and protein binding of intravenous ibuprofen in the premature newborn infant

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Aranda JV, Varvarigou A, Beharry K, Bansal R, Bardin C, Modanlou H, Papageorgiou A, Chemtob S. Pharmacokinetics and protein binding of intravenous ibuprofen in the premature newborn infant. Acta Pædiatr 1997; 86: 289–93. Stockholm. ISSN 0803–5253

The elimination, disposition and protein binding of ibuprofen (IBU) in premature infants were studied for use in the prevention of intraventricular hemorrhage and closure of patent ductus arteriosus. The kinetic profile of i.v. IBU lysine (10 mg/kg bolus) given within the first 3 h after birth was studied in 21 premature neonates (mean birthweight = 944.7 g, range: 575–1450 g; gestational age: 26.8 weeks, range: 22–31 weeks). Blood samples (0.3 ml/sample) were obtained at time 0 and at 1, 3, 6, 12, 24, 48, and 72 h post-dose for IBU by high-performance liquid chromatography (HPLC). Kinetic analyses assumed applicability of one open-compartment model and calculations from the model-independent areas under the time concentration curve (AUC). Data (mean ± SEM) show that apparent volume of distribution (AVd) was 62.1 ± 3.9 ml/kg, plasma $t_{1/2}$ beta was 30.5 ± 4.2 h, elimination rate constant (k_{el}) was 0.032 ± 0.004 h^{-1}, plasma clearance was 2.06 ± 0.33 ml/kg/h and plasma concentration (Cp) at 1 h was 180.6 ± 11.1 mg/l. Gestational age and birthweight were not related to drug elimination. In 10 neonates, IBU maintenance dose of 5 mg/kg once daily on days 2 and 3 generated mean Cp of 116.6 ± 54.5 mg/l and 113.6 ± 58.2 mg/l, respectively. Protein binding by ultrafiltration and capillary electrophoresis showed that the percentage bound IBU was significantly lower in full term cord plasma (94.98 ± 0.39%, $n = 26$) compared to adult plasma protein (mean ± SE = 98.73 ± 0.31%, $n = 8$, $p < 0.0001$). Compared to data from adults and older children, IBU elimination is markedly prolonged in neonates and protein binding is slightly lower. Thus, investigational and clinical therapeutic regimens should be adjusted to account for decreased drug disposition to ensure safe and effective therapy. □ *Cyclooxygenase blocker, ibuprofen, intraventricular hemorrhage, newborn, pharmacokinetics*

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Ibuprofen, one of the most commonly used analgesic, antipyretic, and anti-inflammatory drugs available over the counter, is a cyclooxygenase blocker (1) and decreases the synthesis of prostanoids, particularly vasodilator prostaglandins (2). Recently, we have shown that in contrast to indomethacin and other cyclo-oxygenase blockers, ibuprofen may increase the range of blood pressure at which cerebral blood flow is autoregulated (2, 3) but has little effect on CBF during normotension (4). Like indomethacin, however, it may produce early closure of the ductus arteriosus and may possibly offer cerebrovascular protection. Intravenous ibuprofen has been suggested to decrease the incidence of patent ductus arteriosus if given early in the premature newborn (5). There are as yet no available data concerning the pharmacokinetic disposition of ibuprofen in the premature and newborn infant. Because of its possible role in neonatal therapeutics, the pharmacokinetics of ibuprofen were studied in a group of preterm infants. This study was designed to characterize the pharmacokinetic profile of intravenous ibuprofen in very low birthweight neonates, to be used for future clinical trials in the premature newborn infant for PDA closure and IVH prevention. We tested the hypothesis that ibuprofen, like most drugs used in the neonates, is eliminated slowly in the premature newborn. We also tested the hypothesis that the plasma protein binding of the IBU in the newborn differs from the adult.

Patients and methods

Twenty one premature newborns with mean birthweight of 944.7 g (range = 575–1450 g) and mean gestational age of 26.8 weeks (range = 22–31 weeks) were included in this study if they suffered no severe perinatal asphyxia, no intraventricular hemorrhage within the first 6 h of life, no maternal infection and no congenital malformations. All premature neonates were antenatally followed in our

prenatal or high risk clinic with fetal serial ultrasounds and fetal age assessments. All infants had cranial ultrasounds within 0–6 h of postnatal life to determine the presence of intraventricular hemorrhage. All deliveries were attended by a staff neonatologist and Apgar scores, cord pH, and the need for assisted ventilation were determined to assess perinatal asphyxia. All mothers received no ibuprofen or other NSAID within 3 days prior to labor. All infants received a dose of ibuprofen lysine given intravenously (Merkle, Ulm, Germany) between 0 and 3 h of age with a dose of 10 mg/kg bolus over a period of 2–3 min using a hand-held syringe to deliver 10 mg/ml volume. This drug was given as part of an on-going trial on the use of ibuprofen for closure of the ductus arteriosus and possible prevention of intraventricular hemorrhage in preterm newborns. This study was approved by the Institutional Medical Ethics Committee, and the mode of therapy was discussed with the parents and informed consent was obtained in each case. All infants had arterial catheters and blood samples were obtained 0.3 ml per sample at 0, 1, 3, 6, 12, 24, 48 and 72 h following the dose. Blood sampling was obtained together with other clinically indicated laboratory tests such as blood gases and glucose. Ten of these 21 babies received an additional 5 mg/kg/day at age 48 and 72 h for blood samples (0.3 ml). Plasma drug concentrations were obtained in these babies 1 h after the intravenous administration of ibuprofen. Plasma ibuprofen (0.1 ml) was used for assay by high performance liquid chromatography employing a technique modified from Aravind et al. (18). The chromatography system consisted of a Waters 2010 Chromatography Manager complete with a 496 computer, a Millennium Multi-system software (version 2.1, Mississauga, Ontario), a model 600E power-line multi-solvent delivery system, a model 996M photodiode array UV detector, and a model 715 ultra WISP (Waters Intelligent Sample Processor), a μBondapak C_{18} column (5 mM) with a μBondapak C_{18} pre-column. The mobile phase consisted of 53% acetonitrile (HPLC grade, Fisher Scientific, Montreal, Québec), and 47% filtered, pyrogen-free, organic-free water. The mobile phase was adjusted to pH 4.0 with glacial acetic acid. The flow rate of mobile phase was 1.5 ml/min and the detector was set at 220 nm.

Ibuprofen, 1 mg/ml (Sigma, St Louis, MO, USA), was used to prepare a stock solution of 100 mg/l. Measured aliquots of this solution were added to human (ibuprofen-free) plasma to yield the ibuprofen concentrations of 0.25, 0.5, 1.0, 2.5, 10.0, 25.0 and 50.0 mg/l. The standards were freshly made each assay day. Isobutyl phenyl acetate was used as an internal standard. A 4% concentration was made in methanol and 1 ml of this solution was added to 10 ml of methanol to prepare a working solution. The internal standard (100 ml) was added to the standards and the samples. The assay sample was prepared using 100 ml of internal standard solution, and 100 ml of cold 0.5 M perchloric acid in methanol. The samples were vortexed for 15 sec, centrifuged in a cold centrifuge (4°C) at 3000 rpm for 15 min and 200 ml of the supernatant of each standard or patient samples was injected directly into the column. The retention times for ibuprofen and the internal standard were 6.0 min and 9 min, respectively. The standard curves were linear for ibuprofen concentration ranging from 2.5 to 50.0 mg/l ($r = 0.999$). The detection limit was 0.20 mg/l. The interassay coefficient of variation was < 5.0%. No interference was noted with drugs used in the neonatal period including furosemide, ampicillin, gentamicin, vancomycin, caffeine, theophylline, digoxin, phenobarbital and phenytoin.

Data acquisition and calculations of ibuprofen were performed by the Millennium software. An analysis of the ratios of the areas under the ibuprofen curve was divided by the ratios of the areas under the internal standard curves to generate a linear standard curve of area ratio versus concentration. Interpolation from the linear regression line was used to determine the ibuprofen concentrations in patients and quality control samples.

Protein binding

In adults, IBU is highly protein bound. Since fetal and neonatal serum protein-drug binding may differ from adults, we tested the hypothesis that the binding of IBU to neonatal serum albumin is decreased. The fraction of ibuprofen bound in neonatal and adult plasma proteins was determined by ultrafiltration method followed by capillary electrophoresis. The latter technique (capillary electrophoresis) has been used to study protein binding based on different electrophoretic mobilities of molecules of different size (20, 21). In this study, CE was only used to determine free ibuprofen in the ultrafiltrate.

Heparinized blood samples (1.0 ml) were obtained from adult volunteers (age 25–50 y, $n = 8$) and cord blood from full term newborns (gestational age 39–40 weeks, $n = 26$). IBU concentrations ranging from 0 to 200 mg/l were added to 100 ml of the plasma and the protein binding was determined by capillary electrophoresis following ultrafiltration. An aliquot (0.5 ml) of the plasma drug mixture was placed into a disposable centrifree™ micropartition system (Amicon Division, Beverly, MA, USA) which utilized a YMT ultra-filtration membrane (molecular weight cut off *ca* 30 000) and the plasma sample was centrifuged at 2000 *g* in a fixed angle rotor for 15 min. The aliquots of ultrafiltrate and prefiltered plasma were then injected (20 nl) into a capillary electrophoresis instrument (Waters Quanta 4000E). Data were collected with a Millennium 2010 Software. An untreated fused silica capillary tube (50 μ i.d. × 35 cm in length) was used as a separation tube. All analysis were performed with a UV detection at 214 nm, and a hydrostatic injection (9.8 cm height). The running electrolyte was 50 mM sodium phosphate buffer. Sample injection was set hydrodynamically (20 kV) for 10 seconds. Each injection volume was $\gg$10 nl. The retention times for protein bound drug and free drug were 8.5 minutes and 9.0 minutes, respectively. The free drug was measured from the ultrafiltrate. The drug protein binding in

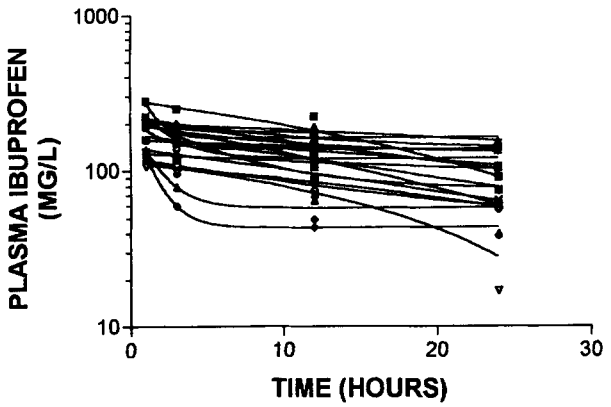

Fig. 1. Plasma disappearance curve of i.v. ibuprofen (10 mg/kg) in 21 preterm newborns. Plasma concentrations (mean ± SEM) at 1, 3, 6, 12 and 24 h were 180.6 ± 49.7, 150.5 ± 45.5, 133.1 ± 41.8, 115.8 ± 35.2, and 94.3 ± 40.7, respectively.

the plasma was calculated from:

$$\text{Percent bound}\,(\%) = \frac{(\text{total–free drug}) \times 100}{\text{total drug in plasma}}$$

The unbound fraction was calculated as the ratio of ibuprofen in the ultrafiltrate to the drug in the plasma. Unbound concentrations of ibuprofen were then calculated as the unbound fraction multiplied by the total drug concentrations in the original plasma sample. The bound concentrations of ibuprofen were calculated as the difference between total and unbound concentration in the original plasma sample.

Analysis of data

The plasma concentrations of ibuprofen were plotted semilogarithmically and analysis of data assumed applicability of the one compartment model. Like most drugs, there is a slight two compartment character of the drug but because of the tremendously long beta half-life of most drugs in the newborn period relative to the alpha phase, this two compartment character of the drug has been shown to be of little clinical consequence for those computations in young children. The elimination rate constant (K_{el}) and the plasma half-life were computed from the slope of the ibuprofen disappearance curve. This slope was determined by the method of least squares. The areas under the time concentration curve (AUC) was calculated by the trapezoidal rule and the apparent volume of distribution (AVd) was computed as dose over AUC × K_{el}. Plasma $t_{1/2}$ was calculated as $0.693/K_{el}$. Clearance was calculated as Dose/AUC. All values were expressed as mean ± SEM.

Results

Pharmacokinetic profile

The disappearance curve of ibuprofen in all infants studied is shown in Fig. 1. Following a dose of 10 mg/kg ibuprofen, there was a slow disappearance curve of this drug from the plasma. Table 1 shows the pharmacokinetic profile of intravenous ibuprofen given at 0–3 h of age in 21 premature newborns. The plasma half-life of ibuprofen from the 21 babies studied was 30.5 ± 4.2 h with an elimination rate constant of 0.032 ± 0.004 h^{-1}. The mean

Table 1. Pharmacokinetic profile of i.v. ibuprofen in premature neonates.

Patients	Birthweight (g)	Gestational age (weeks)	Cp 1 h (mg/l)	AUC	Vd area (ml/kg)	K_{el} (h^{-1})	$t_{1/2}$ (h)	Cl (t) ml/kg/h
1	1005	27.7	280.5	5499.1	36.5	0.04971	13.9	1.82
2	1105	27.57	207	6519.4	50.2	0.03056	22.7	1.53
3	1105	26	132.3	11248.1	76.2	0.01169	59.26	0.89
4	765	27	124.1	1861.0	104.8	0.05122	13.5	5.37
5	575	22.7	158.2	8939.7	63.2	0.01769	39.1	1.11
6	710	25	225.3	6034.0	49.6	0.03340	20.7	1.65
7	815	26.43	275.8	9788.0	39.7	0.02573	26.9	1.02
8	900	26.43	159.6	19305.8	62.9	0.00822	84.3	0.52
9	1155	29	220.9	14515.9	46.2	0.01490	46.5	0.69
10	1065	27.7	184.6	11950.2	55.8	0.01498	46.3	0.83
11	1450	31.14	133.7	4072.9	76.4	0.03209	21.5	2.45
12	595	24	196	3754.6	51.9	0.05125	13.5	2.66
13	610	25.14	202.6	15776.1	49.4	0.01280	54.1	0.63
14	600	22.27	214	3921.4	54.8	0.04648	14.9	2.55
15	635	24	220.5	10718.4	46.9	0.01987	34.8	0.93
16	850	25.43	190.1	11957.5	53.9	0.01549	44.7	0.83
17	1025	26.57	195.4	4281.8	55.2	0.04227	16.3	2.33
18	945	26.57	136	2192.9	84.4	0.05397	12.8	4.56
19	1160	31	107	1790.1	69.6	0.08024	8.6	5.58
20	1410	31.43	113.7	3680.6	88.6	0.03063	22.6	2.71
21	1360	31.43	116.3	3849.5	89.1	0.02913	23.7	2.59
Mean	944.7	26.8	180.6	7698.0	62.1	0.03201	30.5	2.06
SEM	59.9	0.5940	11.1	1095.7	3.9	0.004019	4.2	0.33

Table 2. Developmental aspect of ibuprofen pharmacokinetics.

Age	Dose	Plasma $t_{1/2}$	Author (year)
0–3 h ($n = 21$)	10 mg/kg i.v.	30.5 ± 4.2 h	Aranda (present report)
3 months–10 y ($n = 49$)	8 mg/kg p.o.	1.6 ± 0.7 h	Kauffman, 1992 (6)
6 months–11 y ($n = 18$)	6 mg/kg p.o.	118.2 min (tot) 138.6 min (S+) 88.2 min (R−)	Kelley, 1992 (7)
6–12 y ($n = 19$ CF, $n = 4$ normal)	300 mg p.o. (13 mg/kg)	92 ± 27 min (CF) 86 ± 17 min (normal)	Konstan, 1991 (8)
Adults ($n = 26$)	400 mg p.o	2.2 ± 0.4 h	Albert, 1994 (9, 10)
29.8 ± 6.5 y ($n = 8$)	200–400 mg i.v.	94 min	Martin, 1990 (11)

plasma concentration at 1 h was 180.6 ± 11.1 mg/l and the apparent volume of distribution was 62.1 ± 3.9 ml/kg. The plasma clearance was 2.06 ± 0.33 ml/kg/h.

The 10 newborns who received 5 mg/kg i.v. of ibuprofen as a maintenance dose at day 2 and day 3 showed that the plasma concentrations at day 2 were maintained at 116.6 ± 54.5 mg/l. Similarly, the plasma concentration at day 3 of ibuprofen was 113.6 ± 58.2 mg/l. In these babies, no evidence of PDA was noted, suggesting that maintenance of this plasma concentration of ibuprofen is probably related to ductal closure. There was no correlation between gestational ages at 22–31 weeks between plasma clearance, plasma half-life, and elimination rate constant suggesting that within this fetal age there was no effect of fetal maturity on ibuprofen disposition.

Protein binding

Data from the ultrafiltration and capillary electrophoresis studies show that the percent bound ibuprofen was significantly lower in the cord plasma (mean ± SE: 94.98 ± 0.39%, $n = 26$) compared to the adult plasma protein (mean ± SE: 98.73 ± 0.31%, $n = 8$, $p < 0.0001$). Percent bound IBU at drug concentrations of 42.2 ± 1.9, 76.2 ± 3.5 and 157 ± 19.2 mg/l were 95.0 ± 0.5%, 95.0 ± 0.5% and 94.0 ± 1.5%, respectively (p = ns). IBU protein binding was substrate independent at concentrations of 1–200 mg/l, equivalent to plasma IBU levels after a dose of 10 mg/kg in newborn infants.

Discussion

The elimination of ibuprofen in these premature newborns is substantially slower than that seen in older children and adults (6–17). Table 2 summarizes a comparison of ibuprofen pharmacokinetics during the period of development. In comparison to the young infant of more than 3 months of age, the plasma half-life of ibuprofen is prolonged more than 10-fold compared to the older babies in whom the plasma half-life of ibuprofen is approximately 1.5–2 h.

Ibuprofen is a racemic mixture of R(−) and the more active enantiomer S(+). The R(−)enantiomer is converted to the pharmacologically active S(+)in a unidirectional metabolic inversion (22, 23). Kelley et al. (7) suggested that in febrile children the plasma $t_{1/2}$ of the S(+) enantiomer is longer (138.6 min) relative to the R(−) enantiomer (88 min). Individual kinetic profiles of these enantiomers were not assessed in this study. However, Martin et al. used the same preparation of ibuprofen lysine given to these premature babies in this study in eight adults and found that after a dose of 200–400 mg i.v., the plasma half-life in these adults patients was 94 min, again indicating that ibuprofen is eliminated very slowly in preterm babies.

The prolonged half-life of ibuprofen in the preterm infants may have been due to deficient activity of the hepatic cytochrome P450 hemo-oxygenase complex since the process of hydroxylation and carboxylation contributes to the overall disposition of these drugs. In addition, biotransformation of IBU involves UDP glucuronyltransferase and the activity of many UDPGT isoforms is reduced in the newborn. These factors, including reduced renal function, may contribute to the prolonged elimination of IBU in these premature neonates. Ibuprofen is metabolized and excreted in the adults far more rapidly than in the preterm babies.

Successful design of clinical trials on ibuprofen in closure of the ductus arteriosus, as well as prevention of intraventricular hemorrhage in the preterm, appears to depend in part on the achievement and maintenance of a given blood or a plasma concentration of the drug during the study period (19). In a phase I trial of ibuprofen in preterm newborns (19), PDA was noted in 7/11 (66%) preterm neonates who received saline at 0–3 h after birth. One dose of ibuprofen given during the first day was associated with PDA in 6/11 neonates. The use of a maintenance dose of ibuprofen over a period of 3 days was associated with maintenance of ductal closure (0/12 babies) and a trend towards a decrease in intraventricular hemorrhage. The plasma concentration of ibuprofen generated with this dose schedule is 2.5 times higher than that generated in adults with chronic arthritis (15). Adult patients who ingested 600 mg of ibuprofen 3 times a day for 2 days generated mean plasma ibuprofen of 44.1 ± 9.3 mg/l (15). It is unclear from the present study whether or not these plasma concentrations are necessary for ductal closure. The lack of efficacy, if the drug is not sustained

during the first 3 days, suggests that these plasma concentrations are probably required for maintenance of ductal closure. These plasma concentrations did not give any adverse effects except for a transient decrease in fractional sodium excretion at day 2 (19).

Data indicate that for a given plasma ibuprofen concentration, more free or unbound drug is present in the newborn resulting in a more intense pharmacologic effect relative to adults. The decreased IBU binding should be accounted for in the use of this drug in newborn infants for possible prevention of patent ductus arteriosus or intraventricular hemorrhage.

These data suggest that an intravenous loading dose of 10 mg/kg followed by a maintenance dose of 5 mg/kg/day i.v. for 2 days maintains plasma concentrations related to ductal closure (19). These data also indicate that ibuprofen is eliminated very slowly in premature neonates. This dose regime may be useful for future controlled studies on ibuprofen in the newborn. Application of these data should help in the design of clinical trials especially in the avoidance of predictable and dose-related toxicity.

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Received Nov. 27, 1995. Accepted in revised form Nov. 22, 1996