Oseltamivir Pharmacokinetics and Clinical Experience in Neonates and Infants during an Outbreak of H1N1 Influenza A Virus Infection in a Neonatal Intensive Care Unit

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Detailed oseltamivir pharmacokinetics have yet to be reported in neonates and infants; this group is at high risk of serious influenza-associated complications. Extrapolation of doses from older patients is complicated by rapid organ and drug-metabolizing enzyme maturation. A pharmacokinetic study has been conducted during an influenza A(H1N1) outbreak in a neonatal intensive care unit. Each included patient provided 4 samples for oseltamivir and 4 samples for its active metabolite oseltamivir carboxylate. A population pharmacokinetic model was developed with NONMEM. Allometric weight scaling and maturation functions were added *a priori* to scale for size and age based on literature values. Nine neonates and infants were recruited. A physiologically parameterized pharmacokinetic model predicted typical day 1 area under the curve (AUC₀₋₁₂) values of 1,966 and 2,484 $\mu\text{g} \cdot \text{h}/\text{liter}$ for neonates and infants of ≤ 37 weeks of postmenstrual age (PMA) and > 37 weeks of PMA treated with 1 mg/kg of body weight and 2 mg/kg, respectively. The corresponding steady-state AUC₀₋₁₂ values were 3,670 and 4,559 $\mu\text{g} \cdot \text{h}/\text{liter}$. Premature neonates treated with 1 mg/kg and term babies treated with 2 mg/kg should have average oseltamivir carboxylate concentrations in a range similar to that for adults treated with 75 mg, corresponding to > 200 -fold above the half-maximal inhibitory concentration (IC₅₀) value for influenza A(H1N1) from the start of therapy.

Influenza causes considerable morbidity and mortality each year among children during the seasonal outbreak. Influenza-associated hospitalizations are substantially higher among younger children, especially those aged < 2 years, and highest among infants under 6 months of age. Hospitalization rates in these age groups are comparable to those in groups, such as older adults and patients with chronic underlying diseases, that are considered to be at high risk for influenza-related complications (28, 29). Although influenza is uncommon in neonates, outbreaks have been described in neonatal intensive care units (NICUs) and severe cases have been reported (33, 26, 17).

Oseltamivir is currently recommended for prophylaxis and treatment of confirmed or suspected influenza among high-risk groups, including children < 2 years of age (7). Randomized controlled trials in adults and children in the outpatient setting have shown that early (< 48 h) initiation of neuraminidase inhibitors reduces the duration of illness (18, 31, 32, 12). There are limited data, mainly from studies in adults, suggesting that oseltamivir treatment may reduce influenza-associated complications, such as otitis media, pneumonia, and the risk of death (9, 13, 16, 32, 36). Although children < 1 year of age are at high risk for influenza-associated complications, antiviral medications are not currently approved for this age group, but recommendations for dosing are available (7, 34).

Oseltamivir pharmacokinetics have been reported in a small number of studies in children, but only sparse data exist with regard to infants and neonates (19, 15). In the only study conducted among premature babies, only one sample per patient was collected and data analysis consisted mainly of overlaying measured concentrations on those from older children (3). In order to make dose recommendations, a pharmacokinetic model which

links dosing history with serial concentration measurements is required. Once this relationship is established, the model can be used to recommend a dose.

Oseltamivir is a prodrug for the neuraminidase inhibitor oseltamivir carboxylate, which acts by inhibiting the release of newly formed virions from infected cells and by blocking viral entry into uninfected host cells (20). There appears to be no clear dose-response relationship in viral titers in adults treated with doses ranging from 20 to 200 mg twice daily (10), and the approved adult treatment dose of 75 mg twice daily maintains concentrations exceeding the half-maximal inhibitory concentration (IC₅₀) values for all tested influenza strains by at least 50-fold (11). Oseltamivir carboxylate accumulates in the lung, middle ear, and nasal mucosa at concentrations higher than those measured in the blood in animal studies (11).

The metabolism of oseltamivir to oseltamivir carboxylate is mediated primarily by human carboxylesterase 1 (HCE1), which is expressed predominantly in the liver (27, 35, 2). The expression of HCE1 increases rapidly over the first year of life, indicating that neonates may produce smaller amounts of the active metabolite. In addition, neonates may exhibit decreased renal clearance of drugs and their metabolites, and variations in oral bioavailability

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FIG 1 Schematic diagram of the final model based on Rayner et al. (22). Ka, absorption rate constant; Fm, fraction going to metabolite on the first pass; CLU, unchanged osetamivir clearance; VD, osetamivir volume of distribution; CLTM, central clearance of osetamivir to osetamivir carboxylate; Kam, osetamivir carboxylate appearance rate; CLM, clearance of osetamivir carboxylate; VDM, volume of distribution of osetamivir carboxylate. FM is the fraction converted to metabolite during the first pass, derived from the hepatic well-stirred model. Compartment 1 ([1]) is the depot where the drug is absorbed from the gastrointestinal tract. Compartment 2 ([2]) is the parent drug compartment. Compartment 3 ([3]) is an empirical transit compartment that caused a delay in the metabolite formation rate and, therefore, flip-flop kinetics. Compartment 4 ([4]) is the metabolite compartment.

may also influence drug absorption (3). The neonatal period represents the time at which pharmacokinetic maturation is evolving most rapidly, making it important to collect appropriate data to support dosing recommendations.

During a recent influenza A(H1N1) outbreak in the NICU of P. & A. Kyriakou Children's Hospital, Athens, Greece, a number of preterm and term neonates and infants received osetamivir treatment or prophylaxis. This provided the opportunity to collect serial blood samples during osetamivir dosing and to study its pharmacokinetics.

MATERIALS AND METHODS

Patient recruitment. The NICU is a level 3 unit which serves as a referral center for the greater Athens area and central and southern Greece. Its population often consists of premature neonates and infants with significant comorbidities, including congenital heart disease.

During the 5 days prior to manifestation of the outbreak, 3 out of 33 staff nurses developed laboratory-confirmed influenza A(H1N1) 2009 while another 5 had influenza-like illness (ILI). A total of 22 neonates and young infants were hospitalized in the NICU at the time when the outbreak manifested, and only the patients who required intensive care, and therefore routinely had venous or arterial access for sampling, were enrolled in the pharmacokinetic study.

Dosing was based on postmenstrual age (PMA) and modified by weight according to the recommendations by Acosta et al. (3) For neonates of ≤ 37 weeks of PMA, 1 mg/kg of body weight every 12 hours was used for treatment and prophylaxis; for patients of > 37 weeks of PMA, 3 mg/kg was used every 12 hours for treatment and every 24 hours for prophylaxis. Osetamivir capsules were opened, and the contents were suspended in water per the manufacturer's instructions. All babies received the drug through a nasogastric tube (NGT). Serial blood samples for the pharmacokinetic assay were drawn at predose and then at 1, 4, and 8 h after osetamivir administration. The samples were centrifuged at 3,000 rpm, and the supernatants were stored at -80°C until analyzed. The study was approved by the Hospital Ethics Committee, and written informed consent was obtained from the parents of the participating infants.

Measurement of osetamivir and osetamivir carboxylate levels. Plasma was extracted immediately postsampling and stored at -80°C . Analysis was undertaken within 4 months of storage, which is within the time frame of stability provided by Roche (405 days at -70°C). Osetamivir and osetamivir carboxylate were determined in 50.0- μl aliquots of $\text{K}_2\text{-EDTA}$ plasma using stable-isotope-labeled internal standards for both analytes. The analytes and the internal standards were isolated from

FIG 2 Maturation function fitted to the HCE1 data of Yang et al. (35). Estimated values for the Hill equation were as follows: PM_{50} (postmenstrual age to reach 50% of the adult value), 86.1 weeks; Hill coefficient, 3.17.

plasma samples by protein precipitation and determined by high-performance liquid chromatography (HPLC) with tandem mass spectrometric (MS/MS) detection. Detection was accomplished utilizing turbo ion spray MS/MS in positive-ion multiple-reaction-monitoring mode (MRM). The lower limit of quantification was 1.00 ng/ml with a calibration range up to 250 ng/ml for osetamivir, and the lower limit of quantification was 10.0 ng/ml with a calibration range up to 10,000 ng/ml for osetamivir carboxylate. All measurements were performed at PRA International, Early Development Services, Bioanalytical Laboratory, Assen, The Netherlands.

Pharmacokinetic modeling. For logistic reasons, pharmacokinetic samples were collected after various numbers of prior doses, and subjects received different doses depending on age. For this reason, the data were analyzed using the population pharmacokinetic approach. Doses and concentration measurements were transformed to molar units assuming relative molecular masses of 312.40 g/mol for osetamivir and 284.35 g/mol for the carboxylate metabolite. The model implemented in this study was based on a previously published pharmacokinetic model of osetamivir and osetamivir carboxylate using rich data from 96 adults (22). This model used a two-compartment disposition for osetamivir and a three-compartment disposition for osetamivir carboxylate. As relatively little data were collected in the present study, the model was simplified to give single-compartment dispositions for the parent and metabolite. The model allows for osetamivir formation both during first-pass absorption and from circulating osetamivir and, crucially, for a low rate of metabolite formation. A schematic representation of the model is shown in Fig. 1.

Parameters were scaled *a priori* in order to account for expected maturation and parameter differences related to body size using the method proposed by Tod et al. (30). This method states that in the absence of prior information, pharmacokinetic parameters relating to clearance should follow allometric scaling of $\text{weight}^{0.75}$ and volume parameters scaled with linear weight. Furthermore, in studies involving children aged < 2 years, logistic maturation functions should be estimated or fixed according to literature values to account for age. For this reason, all volume parameters were scaled with linear weight, and clearance of unchanged osetamivir and osetamivir carboxylate was scaled with a renal maturation model published by Rhodin et al. (23). For the intrinsic clearance of osetamivir to osetamivir carboxylate, weight scaling used a power of 0.75. Data from a study investigating developmental expression of human carboxylesterase 1 (HCE1) were extracted from a paper by Yang et al. (35), and a sigmoid maturation function was fitted to relate postmenstrual age with enzyme expression as a fraction of the typical adult value (Fig. 2). Liver blood flow was scaled allometrically by $\text{weight}^{0.75}$ using an assumed 70-kg-adult value of 75 liters/h (21). The well-stirred model for hepatic clear-

TABLE 1 Summary of included patient characteristics^a

Patient no.	PNA (days)	GA (wks)	PMA (wks)	Weight (g)	Oseltamivir dose (mg/kg)	Dose frequency	No. of preceding doses	Clinical diagnosis	Feeding	Trough oseltamivir carboxylate level (nM)
1 ^b	50	29	36	2,790	3	Every 12 h	6	Prematurity, RDS, PDA, PFO	NGT	1,695
2	86	24	36	1,460	1	Every 12 h	6	Prematurity, CLD, PDA, IVH	NGT	601
3	86	24	36	2,370	1	Every 12 h	6	Prematurity, NEC, <i>Staphylococcus sepsis</i> , IVH, seizures	Bottle + NGT	139
4	12	28	29	1,220	1	Every 12 h	6	Prematurity, RDS	NGT	1,825
5	2	28	28	1,440	1	Every 12 h	4	Prematurity, RDS	TPN	819
6	58	36	44	3,350	3	Every 24 h	3	Prematurity, esophageal atresia, IUGR	NGT + TPN	125
7	34	25	29	1,320	1	Every 12 h	4	Prematurity, NEC, jejunostomy, PDA, <i>Staphylococcus sepsis</i> , PVL	TPN	644
8	10	40	41	2,370	3	Every 24 h	2	Congenital heart disease, NEC	TPN	1,111
9	84	40	52	3,300	3	Every 24 h	2	Congenital heart disease, <i>Staphylococcus sepsis</i>	NGT + TPN	352

^a PNA, postnatal age; GA, gestational age; PMA, postmenstrual age (GA plus postnatal age); NEC, necrotizing enterocolitis; NGT, nasogastric tube; bottle + NGT, bottle and nasogastric tube; TPN, total parenteral nutrition; PDA, patent ductus arteriosus; PVL, periventricular leukomalacia; IVH, intraventricular hemorrhage; IUGR, intrauterine growth retardation; CLD, chronic lung disease; PFO, patent foramen ovale; RDS, respiratory distress syndrome.

^b Patient with influenza.

ance was used to estimate the central oseltamivir clearance to the metabolite (CLTM) and the fraction converted to the metabolite on the first pass (FM) by estimating a single intrinsic clearance parameter that was scaled with weight and the HCE1 maturation function (Fig. 2). The equation used for the well-stirred model was $CLTM = (CLI \times FQ)/(CLI + FQ)$, where CLI is intrinsic clearance and FQ is liver blood flow.

Nonlinear mixed-effect model fitting was undertaken using the Laplace approximation method in NONMEMVII (4). Interindividual variability was explored for all parameters, and data below the limit of quantification were included using the M3 method, which retains all measured samples regardless of whether a concentration was reported and uses the undetectable samples by estimating the probability that they were indeed below the detection limit (5). Model diagnostics include plotting observed concentrations versus model predictions, objective function value (OFV) assessments, conditional weighted residuals versus time after dose, and a prediction-corrected visual predictive check (PCVPC) (6). Parameter uncertainty was assessed with a nonparametric bootstrap. The final model was used to simulate expected day 1 and day 7 concentrations given the current treatment dosing recommendations by Acosta et al. (3) and also using a fixed dose of 2 mg/kg for all subjects of >37 weeks of PMA. Demographics were generated from a database of neonatal and infant values, including those of the present study, that contained 1,000 demographic sets. Ten simulation subproblems were used, giving a simulated data set of 10,000 individuals. This database contained demographics with a gestational age (GA) range of 24 to 41 weeks and a postnatal age (PNA) range of 1 to 92 days. Pharmacokinetic parameters were generated from these simulations in order to compare with published values.

RESULTS

Patient recruitment and clinical details. Of the 22 hospitalized neonates and infants at the time of outbreak, all 9 who required intensive care were recruited to the oseltamivir pharmacokinetic study; demographic and clinical characteristics for these 9 patients are presented in Table 1. Oseltamivir treatment was administered to one neonate with influenza A(H1N1) 2009 infection documented by reverse transcription-PCR (RT-PCR) (patient 1), while the remaining 8 patients received prophylaxis. All patients had normal serum creatinine concentration and renal function. Three neonates were receiving total parenteral nutrition (TPN) and no enteral feeding, and three neonates were suffering from necrotizing enterocolitis (NEC) (Table 1). Average oseltamivir carboxylate concentrations estimated from the raw data for the

three patients with NEC were 360 and 681 nmol for the two receiving 1 mg/kg and 1,715 nmol for the subject receiving 3 mg/kg.

During the outbreak, three neonates (all born preterm) out of the original 22 neonates developed influenza A(H1N1) 2009 and received oseltamivir treatment, while 19 patients received prophylaxis. Two patients manifested respiratory symptoms, and influenza infection was confirmed by RT-PCR. One participated in the pharmacokinetic study (patient 1), and the second was a neonate of 45 weeks of PMA, born at a GA of 35 weeks, who developed only an upper respiratory tract infection and had an uneventful course. The third patient was the triplet of patients 2 and 3 (Table 1) who developed influenza on the 5th day of oseltamivir prophylaxis. This patient (GA, 24 weeks; PNA, 84 days) had chronic lung disease, had recently been weaned off oxygen, and was being nursed in a high-dependency unit (HDU). Oseltamivir prophylaxis (1 mg/kg every 12 hours) was started without prior influenza testing since she was not intensive-care dependent and there were no clinical or laboratory signs of infection. On the 5th day, the patient developed tachypnea, apnea, and oxygen desaturation. RT-PCR was performed, and influenza A(H1N1) 2009 was confirmed. Oseltamivir dosing was increased to 3 mg/kg every 12 hours. Her condition deteriorated further, requiring, in addition to supplemental oxygen, respiratory support with nasal continuous positive airway pressure (CPAP) for 3 consecutive days. A chest X ray revealed bilateral interstitial infiltrates. A second PCR performed after 5 days of full treatment remained positive for H1N1, and the strain was susceptible to oseltamivir. Oseltamivir treatment was prolonged to a total of 10 days, and repeated PCR testing at the end of the extended oseltamivir course was negative for H1N1.

Close clinical and laboratory monitoring revealed that none of the 22 patients who received oseltamivir treatment or prophylaxis developed any serious adverse effects. One patient (patient 9) developed diarrhea and dehydration on the 9th day of oseltamivir prophylaxis, which was discontinued. At the same time, she was also receiving broad-spectrum antibiotics for sepsis. A second infant (patient 3) had a 2-fold elevation of liver function tests which returned to normal without treatment interruption.

Pharmacokinetic modeling. Each of the 9 recruited patients

FIG 3 (A) Raw data, with solid lines connecting oseltamivir carboxylate concentrations and dashed lines connecting oseltamivir concentrations. (B) Population level model predictions (PRED). (C) Population-predicted concentrations plotted against observed concentrations (Obs conc). Pred Corr, prediction corrected. (D) Conditional weighted residuals (CWRES) plotted against time after dose (TAD).

provided 4 samples each for oseltamivir and oseltamivir carboxylate during steady-state dosing, giving 72 concentration measurement points in total. No oseltamivir carboxylate measurement was below the limit of quantification, but 6 of the oseltamivir concentrations (5 troughs and one 8-h sample) were undetectable. The raw data showed a large degree of between-subject variability with a >10 -fold range in observed oseltamivir trough concentrations (Table 1). The simplified Rayner (22) model (Fig. 2) fitted the data well (Fig. 3), although numerical instability meant that not all parameters could be estimated. The volumes of distribution of the parent (VD) and metabolite (VDM) were fixed to 91 and 25.6 liters/70 kg, respectively (11), and a constraint was placed to stop the oseltamivir carboxylate formation rate (Kam) from exceeding the absorption rate constant for oseltamivir and oseltamivir carboxylate (Ka). No difference in fitness was seen when the clearances of the unchanged parent and metabolite (CLU and CLM, respectively) were assumed to take the same value. The estimated mature value for intrinsic clearance (95% confidence interval [CI]) was 3,284 (2,071 to 4,982) liters/h, and model parameter estimates are given in Table 2.

The visual predictive check (Fig. 4) showed that the median model-simulated data were similar to those observed. Simulations from the final model showed that the Acosta et al. (3) dosing regimen of 1 mg/kg for children of ≤ 37 weeks of PMA produces day 1 concentrations in a range similar to, if slightly higher than, that observed in adults (Table 3 and Fig. 5A). For infants and neonates of >37 weeks of PMA, the 3-mg/kg dosing regimen pro-

TABLE 2 Parameter estimates and 200 sample bootstrap results from the final model^a

Parameter	Estimate	Mean bootstrap estimate	5th percentile from bootstrap	95th percentile from bootstrap
Ka (/h)	0.22	0.21	0.098	0.39
VD (liters/70 kg) ^b	90.7	90.7	90.7	90.7
CLU and CLM (liters/h/70 kg)	30.1	30.2	20.6	45.4
Kam (/h)	0.034	0.031	0.021	0.063
VDM (liters/70 kg) ^b	25.6	25.6	25.6	25.6
CLI (liters/h/70 kg)	3,284	3,222	2,071	4,982
CLTM (liters/h/70 kg)	73.3	73.3	72.4	73.9
Proportional residual error on parent (%)	54.3	51.4	26.1	70.2
Proportional residual error on metabolite (%)	23.2	21.7	13.4	28.8
BSV for Ka (% CV)	57.5	54.3	28.7	82.4
BSV for CLU and CLM (% CV)	59.4	54.1	32.5	72.2
BSV for CLI (% CV)	71.0	65.9	35.2	96.7

^a Ka, absorption rate constant for oseltamivir and oseltamivir carboxylate; CLU, renal clearance of unchanged oseltamivir; VD, volume of distribution of oseltamivir; Kam, conversion rate of oseltamivir to oseltamivir carboxylate; CLI, intrinsic clearance; CLTM, central oseltamivir clearance to metabolite—not an estimated parameter but derived from the well-stirred hepatic model; CLM, clearance of oseltamivir; VDM, volume of distribution of oseltamivir carboxylate; BSV, between-subject variability; % CV, percent coefficient of variation.

^b Fixed value.

FIG 4 Prediction-corrected (Pred Corr) visual predictive checks (6) for oseltamivir and oseltamivir carboxylate. The solid lines are the medians of the observed data. The dashed lines are the medians of the model-simulated data. The shaded areas are the 95% confidence intervals around the median simulated lines.

duces higher day 1 area under the curve from 0 to 12 h (AUC_{0-12}) than those seen with adults and older children and 2 mg/kg appears sufficient (Table 3 and Fig. 5B).

DISCUSSION

A rigorous oseltamivir pharmacokinetic study has been conducted in the challenging environment of an influenza outbreak on a level 3 NICU. The key finding was that despite a large degree of between-subject variability observed, it appears that the typical oseltamivir concentrations achieved with the Acosta et al. (3) dosing regimen of 1 mg/kg yield day 1 AUC_{0-12} and average concentration (C_{ave}) values for preterm neonates of ≤ 37 weeks of PMA that are similar to those observed in older subjects. For older ne-

onates and infants, Acosta et al. (3) recommend 3 mg/kg, whereas the standard adult dose is 75 mg (approximately 1 mg/kg). While it is known that drug clearance is higher on a mg/kg basis in infants than in adults, the question of whether infants under 1 month of age should receive 3-fold the adult dose needs to be raised. Our simulations show that 2 mg/kg is adequate (Table 3 and Fig. 5B) for those of >37 weeks of PMA and that 1 mg/kg is adequate for those of ≤ 37 weeks of PMA (Table 3 and Fig. 5B). While comparisons with studies in older subjects are complicated by the fact that noncompartmental pharmacokinetic analysis has been used, making extrapolations to first-dose and steady-state conditions difficult, there are two key comparisons that stand out. First, the neonatal data seem more variable than published adult data

TABLE 3 Summary of the median simulated oseltamivir carboxylate AUC_{0-12} s, trough concentrations, and average concentrations of the first dose on day 1 and day 7 of treatment^a

Dosing amount or source and patient group	Day 1			Day 7		
	AUC_{0-12} [nM · h (μg · h/liter)]	C_{12} [nM (μg/liter)]	C_{ave} [nM (μg/liter)]	AUC_{0-12} [nM · h (μg · h/liter)]	C_{12} [nM (μg/liter)]	C_{ave} [nM (μg/liter)]
Acosta et al. dosing						
PMA > 37 wks, 3 mg/kg every 12 h	12,999 (3,696)	968 (275)	1,083 (308)	23,562 (6,700)	1,340 (381)	1,963 (558)
PMA ≤ 37 wks, 1 mg/kg every 12 h	6,935 (1,972)	610 (173)	578 (164)	13,110 (3,728)	825 (235)	1,092 (311)
PMA ≤ 37 wks, PMA > 32 wks, 1 mg/kg every 12 h	5,749 (1,635)	455 (130)	479 (136)	10,240 (2,912)	611 (174)	853 (243)
PMA ≤ 32 wks, 1 mg/kg every 12 h	7,760 (2,207)	707 (201)	647 (184)	15,002 (4,266)	954 (271)	1,250 (355)
2-mg/kg dosing						
PMA > 37 wks, 2 mg/kg every 12 h	8,814 (2,506)	654 (186)	735 (209)	15,854 (4,508)	907 (258)	1,321 (356)
Literature data						
Children aged <2 years in CASG 114 (3), 3 mg/kg every 12 h	Not reported	Not reported	Not reported	15,214 (4,326) ^b	Not reported	1,268 (361) ^b
Caucasian adults, 75 mg every 12 h (25)	3,840 (1,092)	Not reported	320 (91)	7,983 (2,270)	464 (132)	665 (189)
Japanese adults, 75 mg every 12 h (25)	4,807 (1,367)	Not reported	401 (114)	8,004 (2,276)	503 (143)	667 (190)
Adults, 75 mg ^c every 12 h (11)	6,429 (1,828)	Not reported	536 (152)	10,466 (2,976)	Not reported	872 (248)

^a Using the dosing recommendations of Acosta et al. (3), compared with the values reported by Acosta et al. (3), Schentag et al. (25), and He et al. (11). Values are presented in molar units to compare with IC_{50} s, and values in brackets are in $\mu\text{g} \cdot \text{liter}^{-1}$ to facilitate comparisons with other pharmacokinetic reports. C_{12} , trough concentration; C_{ave} , average concentration.

^b Steady-state values are reported and assumed to be similar to day 7 values.

^c Midpoint of 50-mg and 100-mg doses, taken from Table 5 in He et al. (11).

FIG 5 (A) Box plots showing the simulated oseltamivir carboxylate AUC_{0-12} values for ≤ 37 -week-old infants receiving 1 mg/kg every 12 hours on day 1 and day 7 of treatment. The dashed lines give the highest reported adult AUC_{0-12} s from 75-mg dosing that were reported by He et al. (11) for comparison. (B) Box plots showing the simulated oseltamivir carboxylate AUC_{0-12} values for >37 -week-old infants receiving either 2 or 3 mg/kg every 12 hours on day 1 and day 7 of treatment. The dashed lines give the highest reported adult AUC_{0-12} s from 75-mg dosing that were reported by He et al. (11) and the solid lines give the reported steady-state AUC_{0-12} s from children aged <2 years in the CASG 114 study (3) for comparison.

(trough concentrations observed in the current study varied 10-fold), but this is probably to be expected given the smaller dosing volumes, the fact that the neonates were acutely ill, and the fact that variable numbers of preceding doses had been administered. The second point is that despite some patients in this study suffering with NEC, receiving the drug by nasogastric tube, and hav-

ing supposedly lower expression of HCE1, all had measurable oseltamivir carboxylate levels commensurate with therapeutic effect. The rapid estimated intrinsic clearance value meant that the overall CLTM approached the liver blood flow (Table 2), so it is clear that carboxylation of oseltamivir is a highly efficient process and, as a result, developmental differences in enzyme expression have little impact on the amount of metabolite formed.

One further difference in the pharmacokinetic model parameter estimates between this study and the adult model (22) is the apparently slower metabolite formation rate seen in neonates and infants which means higher per-kg doses are required to achieve day 1 concentrations similar to those of adults. In healthy adults, the half-life of oseltamivir carboxylate when given alone is similar to that of oseltamivir, but when the carboxylate metabolite is measured following oral oseltamivir dosing, its half-life is significantly longer (11). This phenomenon is known as flip-flop kinetics, whereby the metabolite formation rate is slower than its elimination rate, meaning the rate of disappearance from the body is governed by the rate of formation. The estimate for the oseltamivir carboxylate formation rate (K_{am}) was 0.034/h, which gives a mean absorption time of 29.4 h and is longer than the adult model (22) value of 0.121/h, equating to a mean absorption time of 8.3 h. A possible reason for this is that neonates in this study were all in intensive care and 4 received total parenteral nutrition, which can lead to cholestasis and, therefore, potentially slower metabolite appearance. In Table 3, it can be seen that predicted day 1 concentrations with 1 mg/kg for infants of ≤ 37 weeks of PMA and 2 mg/kg for older patients will give AUC_{0-12} values close to but slightly higher than those seen in adults. Reported *in vitro* IC_{50} s for H1N1 are between 0.7 and 2.2 nM (8), the typical average concentrations predicted by the model were around 200-fold higher than this value, and the minimum oseltamivir carboxylate concentration measured in the raw data for this study was >50 -fold higher (Table 1). The tendency to increase doses of oseltamivir to minimize the emergence of drug-resistant influenza needs to be tempered by the fact that there is no clear relationship between oseltamivir dose and viral titers (10) in the range of 20 to 200 mg in adults. The dosing of 1 mg/kg for babies of ≤ 37 weeks of PMA and 2 mg/kg for all older neonates and infants gives typical day 1 concentrations that marginally exceed adult 75-mg dosing and should provide adequate concentrations for maximal activity without unduly increasing adverse reactions from overdosing.

The data analysis approach taken in this study was to use non-linear mixed-effects modeling. This differs from previous pediatric studies (19) in which noncompartmental description of the data was performed by reporting observed values, such as the AUC, maximum concentration of the drug in serum (C_{max}), and time to maximum concentration of the drug in serum (T_{max}). In addition to this, while most noncompartmental analyses have reported the AUC and C_{max} (19, 11, 25), because oseltamivir carboxylate works by inhibiting virion release, we hypothesized that trough and average (taken as AUC_{0-t}/time) concentrations would be more relevant for efficacy. The reason for taking the population approach in this study was that firstly, the data could not be assumed to all be at steady state because, for technical reasons, patients had differing numbers of previous doses before pharmacokinetic sampling. Second, patients received different doses because of differing ages, and so merely reporting observed pharmacokinetic parameters would not have been useful. Instead, a model-based approach incorporating size scaling and maturation

functions (30) allowed for the estimation of model parameters linking size and age with dose to predict concentrations. Using a structural model similar to that previously reported with rich data in adults (22), a model has been developed that gives a good description of the observed data (Fig. 3) and also simulates data with a median trend that does not significantly differ from that of the observed concentrations (Fig. 4). These model diagnostics give confidence that the simulations performed will give a good reflection of typical observed day 1 and day 7 concentrations (Table 3 and Fig. 5), and the use of physiologically plausible size and maturation functions (30) means that this model can also be used for analyzing oseltamivir pharmacokinetics in older children. If such data were added, it should be possible to better investigate the rate of maturation of the oseltamivir carboxylate formation rate.

While none of the neonates on prophylaxis in this pharmacokinetic study developed H1N1, as mentioned in the Results, one other patient on the ward receiving prophylaxis did test positive for A(H1N1) 2009. As PCR testing was not performed prior to prophylaxis initiation in this patient, it is not possible to discern the point at which infection occurred, but given that symptoms started 5 days after oseltamivir commenced, it is likely that this represents a case of failed prophylaxis. From the pharmacokinetic data in the other neonates, including this patient's two siblings, it seems unlikely that this possible prophylaxis failure was due to inadequate oseltamivir concentrations. As seen in Table 1 and Fig. 3, even in the 3 patients with necrotizing enterocolitis (NEC), levels of oseltamivir carboxylate were similar to those of patients without gastrointestinal pathology that could compromise absorption. Following a dose increase and supportive care, the patient did recover to become free of H1N1, but how much of this was due to oseltamivir and how much was due to the supportive care cannot be judged.

A possible criticism of this study is the small number of subjects included. Rather than use the population approach to analyze randomly collected data in large numbers of subjects, this study concentrated on collecting good-quality full-profile data, which necessitated a single center and rigorous collection of dosing and sampling history. This, along with the facts that there had to be an influenza outbreak (it would be unethical to dose and sample from neonates who have no clinical indication for oseltamivir treatment) and that only children who had routine line access to use for sampling could be recruited to the pharmacokinetic arm, meant only 9 subjects were recruited. With 9 subjects, there are clearly insufficient data to estimate covariate parameter relationships (24), so instead, expected size, organ function, and enzyme maturational scaling methods were used to ensure parameters could be compared across age groups. By fixing liver blood flow, the relationship between age and weight and renal function, maturation of HCE1, and principles of scaling for body size with allometry, the data borrow strength from known physiology to increase the credibility of dosing extrapolations. Furthermore, during model building, rationalization was achieved by fixing the volume of distribution and making unchanged and metabolite clearance equal, so only 4 fixed effects were estimated. Extracellular fluid volume can be approximately 25% higher in neonates than in adults (14), and so the model was reestimated with a 25%-higher fixed VDM. This made no change in the CLU and CLM estimates and a <1% change in the CLI estimate, so the model fit is clearly unaffected by assuming different values of VDM, probably due to the flat profiles which give little information on vol-

ume parameters. A further rationalization of the model is that we did not estimate a separate bioavailability parameter. Rather, we used the extraction ratio derived from the well-stirred model to be the fraction converted at first pass, thereby obviating the need to estimate CLTM and FM separately.

In many ways, this study reflects the philosophy of mathematical rather than statistical modeling in biology; it has captured the typical trend that hints, for example, at 2 mg/kg being the most appropriate dose for infants (Fig. 5B). By not only relying on the data collected, as with a pure top-down approach, the model incorporates mechanistic covariates and scaling that places it in the middle of the hierarchy of pharmacokinetics between purely empirical and fully physiological (1). In doing so, it borrows strength from both the observed data and established biological processes, and it is envisaged that this should give more confidence to the dosing guidelines produced. Now that the structural model and its biological scaling have been established, further pharmacokinetic data, particularly if they also include measures of clinical response, will enhance our understanding of the variability in oseltamivir pharmacokinetics and therefore aid in defining the optimum dose.

In conclusion, the pharmacokinetics of oseltamivir in 9 neonates and young infants has been described, and the 1-mg/kg twice-daily dose in patients of ≤ 37 weeks of PMA and the 2-mg/kg twice-daily dose in older neonates and infants give oseltamivir carboxylate concentrations similar to those seen during standard adult dosing of 75 mg. These concentrations vastly exceed the IC_{50} for H1N1 and therefore are expected to produce the maximum possible therapeutic effect. No serious adverse effects were observed among the treated infants, and those who developed influenza recovered uneventfully.

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