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Raltegravir In Vitro Effect on Bilirubin Binding

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Abstract

Drugs that displace bilirubin from albumin may increase the risk of kernicterus in neonates. We evaluated the effect of raltegravir on bilirubin-albumin binding in pooled neonatal serum using the peroxidase method. Raltegravir had minimal effect on bilirubin-albumin binding at concentrations of 5 and 10 μ M, caused a small but statistically significant increase in unbound bilirubin at 100 μ M, and caused potentially harmful increases at 500 and 1000 μ M. Our data suggest that the effect of raltegravir on neonatal bilirubin binding is unlikely to be clinically significant at typical peak concentrations reached with usual dosing.

Keywords

raltegravir; bilirubin binding; neonate

While many antiretroviral drugs are available for use in adults and older children, the number available for use in neonates is limited due to a lack of neonatal safety and dosing information as well as lack of formulations appropriate for newborns.(1) There is an urgent need for additional antiretroviral agents that can be used in neonates for both HIV prophylaxis and treatment. Raltegravir, the first HIV integrase inhibitor to be licensed by the FDA for use in adults and children $\geq$ 2 years of age, is a potent antiretroviral widely used in the treatment of HIV. Raltegravir is under consideration for use in the perinatal setting to both prevent mother to child HIV transmission as well as to treat neonates diagnosed with HIV infection.

The reports in the 1950's of kernicterus associated with displacement of bilirubin from albumin by sulfisoxazole made clear the potential serious adverse effects of drug-bilirubin interactions.(2, 3) Any drug intended for use in neonates should be evaluated for its potential to displace bilirubin from albumin. Raltegravir is 83% bound to plasma proteins and an assessment of its effect on bilirubin-albumin binding is necessary before its use can be considered in newborns. This study describes an *in vitro* assessment of the effect of raltegravir on bilirubin-albumin binding in pooled, bilirubin-enriched neonatal serum using the peroxidase method.(4) The peroxidase method measures only the non-albumin bound (unbound bilirubin) during the horseradish peroxidase catalyzed oxidation of bilirubin in serum. The albumin-bound bilirubin fraction is protected from oxidation. Drugs such as

benzyl alcohol, sulfisoxazole, and ibuprofen interfere with bilirubin-albumin binding and can increase the unbound bilirubin fraction. The peroxidase method has been used to determine the levels at which drugs cause a significant increase in unbound bilirubin.(5)

MATERIALS and METHODS

Preliminary Study Reagents and Methods

Some drugs (e.g. paraben preservatives) may interfere with the peroxidase method.(8) Therefore, a preliminary study was done examining the effect of raltegravir on the rate constant (Kp) for the peroxidase-catalyzed oxidation of bilirubin by peroxide only.

Horseradish peroxidase (Sigma-Aldrich, St. Louis, MO, 113 PPU/mg), hydrogen peroxide, and bilirubin (Porphyrin Products, Logan, UT) solutions were prepared as previously described.(6) A 1.06 mM solution of raltegravir (Merck) was prepared by dissolving 51 mg in 1.0 mL NaOH, pH 9.0.

To measure the effect of raltegravir on the peroxidase method, 25 μ L of diluted stock peroxidase, 25 μ L of peroxide, and 30 μ L of NaOH, pH 9, or 1.06 mM raltegravir (final concentration) were added to 3.0 mL of 0.1 M phosphate buffer in a thermostated cuvette holder (37°C). Five μ L of bilirubin was then added and the decrease in light absorbance (rate of bilirubin oxidation) at 460 nm was monitored every second for 20 seconds using an HP-8450 spectrophotometer. The Kp was then calculated as the slope of

$\ln\left(\frac{A_{440}}{A_{0440}}\right) = Kp \times \text{time}$, where A440 is the absorbance at each time and A₀₄₄₀ is the initial absorbance.

Compared to Kp (mean $\pm$ SD) for the peroxidase-catalyzed oxidation of bilirubin with raltegravir present (1.33 $\pm$ 0.15 per second), Kp with raltegravir absent (1.48 $\pm$ 0.12 per second) was not significantly different (t=1.59, p>0.05), indicating that raltegravir does not interfere with the peroxidase method.

Main Study Reagents and Methods

Horseradish peroxidase (Sigma-Aldrich), hydrogen peroxide, and sulfisoxazole (Sigma-Aldrich) solutions were prepared as previously described. (5, 6) Raltegravir was prepared as described above. To study the effects of raltegravir and sulfisoxazole on bilirubin displacement, the drugs were added to 1.0 mL aliquots of the bilirubin-enriched neonatal sera to yield reaction concentrations between 5 and 1000 μ M (see below). Pooled neonatal serum (Merck& Co., Inc.) was purchased from Bioreclamation, LLC: The pH was adjusted to 7.4 by adding 0.1 M HCl. Albumin concentrations in the neonatal sera were measured using the bromocresol green method.(7) The pooled serum was enriched with bilirubin to concentrations of $\approx$ 10 mg/dL and $\approx$ 20 mg/dL by adding bilirubin (2 mg/dL in 0.1 M NaOH) and pH readjusted to pH 7.4 with 0.1 M HCl, and contained albumin at concentrations of 3.1 $\pm$ 0.01 g/dL (n=3) and 3.2 $\pm$ 0.07 g/dL (n=3), respectively.

The unbound bilirubin concentration was then measured from the decrease in absorbance at 460 nm using a Global FloPro Spectrophotometer (Global FIA, Inc.) equipped for Zone Fluidics reagent handling.(6) The reaction was carried out at a 1:1 sample dilution by creating a zone of 10 μ L of sample, 4 μ L of peroxide, and 6 μ L of peroxidase reagent.

RESULTS

Table 1 presents the unbound mean bilirubin concentrations with no additional drug added or in the presence of varying concentrations of raltegravir or sulfisoxazole (positive control)

using pooled neonatal serum. Each condition was repeated 6 times at total bilirubin concentrations of 10.7 mg/dL (bilirubin/albumin molar ratio = 0.39) and 18.8 mg/dL (bilirubin/albumin molar ratio = 0.66). At the lower total bilirubin concentration, unbound bilirubin levels were significantly increased (1.14–1.77-fold) at raltegravir concentrations of 100 μ M or greater, but never exceeded those seen with sulfisoxazole at therapeutic concentrations (500 μ M, 1.91-fold). At the higher bilirubin concentration, a small, but statistically significant increase in unbound bilirubin began at 10 μ M (1.10-fold), but unbound bilirubin concentrations did not reach those achieved with therapeutic levels of sulfisoxazole (1.42-fold) until the raltegravir concentration reached 500 μ M (1.35-fold).

DISCUSSION

The rate of HIV transmission from HIV-infected pregnant women to their infants is less than 2% with use of the recommended perinatal treatment regimen of maternal combination antiretroviral therapy during pregnancy. [9] In addition, current HIV diagnostic technology allows diagnosis of HIV to be made within the first weeks of life, and neonates found to be HIV-infected should be started on combination antiretroviral therapy as soon as possible. [10]

While there are now more than 20 antiretroviral drugs in 6 classes approved for use in HIV-infected adults, only a few of these drugs can be used in neonates due to lack of neonatal pharmacokinetic data, concerns over neonatal drug toxicity, and/or lack of appropriate neonatal formulations. [1] There is an urgent need for additional antiretroviral drugs that can be safely and effectively used in neonates. Raltegravir, the first HIV integrase inhibitor approved for clinical use, has been shown to be a potent antiretroviral agent when used as part of combination antiretroviral therapy and is a component of one of the first line treatment regimens for HIV infected adults. Raltegravir is attractive for use in the perinatal setting due to its potency, low toxicity, ability to be formulated as oral granules for suspension suitable for use in neonates and mode of action of prevention of integration of HIV genetic material into host DNA.(9)

Before any drug can be used in neonates, its potential to compete with and displace bilirubin from plasma albumin should be investigated. Raltegravir is 83% bound to albumin in plasma, raising the possibility that it could have a significant effect on bilirubin-albumin binding. Our data demonstrate that at low total bilirubin concentrations (10.7 mg/dL), raltegravir has an effect on bilirubin-albumin binding starting at concentrations of 100 μ M, but unbound bilirubin concentrations do not reach those seen with therapeutic sulfisoxazole concentrations (~500 μ M) even with raltegravir concentrations of 1000 μ M. At higher total bilirubin concentrations (18.8 μ g/dL), raltegravir causes a small but statistically significant increase in unbound bilirubin beginning at 10 μ M and potentially harmful increases at 500 and 1000 μ M. The 95% inhibitory concentration of raltegravir for wild-type HIV is 0.033 μ M. In the initial dose ranging study of raltegravir in HIV-infected adults, standard raltegravir doses of 400 mg twice a day resulted in geometric mean C_{max} raltegravir concentrations of 4.5 μ M, median C_{max} of 6.5 μ M and maximum observed C_{max} of 10.2 μ M.(10) In a study of raltegravir pharmacokinetics in children dosed with approximately 6 mg/kg twice daily as chewable tablets, geometric mean C_{max} values were 11.7 μ M for children ages 2 through 5 years and 12 μ M for children ages 6 through 11 years.(11) Our data thus suggest that the effect of raltegravir on neonatal bilirubin binding is unlikely to be clinically significant unless concentrations 50–100 fold higher than typical peak concentrations are reached.(9) In contrast, sulfisoxazole causes significant and potentially toxic displacement of bilirubin at typical therapeutic concentrations (~500 μ M).

Despite these reassuring bilirubin binding findings, the use of raltegravir in neonates must be investigated cautiously. There may be other components present in the plasma of a particular neonate which may impact bilirubin binding. In addition, the primary route of metabolism of both raltegravir and bilirubin is glucuronidation by the UGT1A1 isoenzyme. (9) UGT1A1 activity is low immediately after birth and increases approximately 100-fold over the first three months of life, with extensive inter-individual variability. (12, 13) Adults with the UGT1A1*28/*28 genotype, associated with Gilbert's syndrome, have a marked reduction in UGT1A1 activity, a tendency to hyperbilirubinemia and raltegravir concentrations 40% higher than those in adults with the wild type UGT1A1*1/*1 genotype. (14) These typical developmental changes in UGT1A1 activity and the potential for genetically decreased UGT1A1 activity in some individuals must be considered when investigating raltegravir dosing in children.

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Table 1

Mean ($\pm$ standard deviation) unbound bilirubin concentrations ($\mu\text{g/dL}$) and fraction of unbound bilirubin (%) with no additional drug or in presence of varying concentrations of raltegravir or sulfisoxazole.

Total Bilirubin (mg/dL)		10.7	18.8
No Drug		3.46 $\pm$ 0.25 (32.3%)	7.60 $\pm$ 0.27 (40.4%)
Raltegravir (μM)	5	3.39 $\pm$ 0.22 (31.7%)	7.80 $\pm$ 0.32 (41.5%)
	10	3.37 $\pm$ 0.18 (31.5%)	8.04 $\pm$ 0.18* (42.8%)
	100	3.95 $\pm$ 0.30* (36.9%)	8.39 $\pm$ 0.71* (44.6%)
	500	4.87 $\pm$ 0.24* (45.5%)	10.28 $\pm$ 0.46* (54.7%)
	1000	6.11 $\pm$ 0.26* (57.1%)	11.22 $\pm$ 0.72* (59.7%)
Sulfisoxazole (μM)	500	6.60 $\pm$ 0.25* (61.7%)	10.77 $\pm$ 0.36* (57.3%)
	1000	8.64 $\pm$ 0.17* (80.7%)	-----

* Significantly greater than no drug (p 0.05), n=6 for each concentration

Typical C_{max} for raltegravir