Protein binding of propranolol and verapamil enantiomers in maternal and foetal serum

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The protein binding of the enantiomers of propranolol and verapamil was measured in 19 pairs of maternal and foetal serum obtained at delivery. The binding of the enantiomers of both drugs was lower in foetal than in maternal serum. In maternal serum the mean ($\pm$ s.d.) unbound percentages were 22.4 ± 6.2 and 20.7 ± 6.6 for R- and S-propranolol, and 16.8 ± 5.5 and 22.5 ± 6.2 for R- and S-verapamil; in foetal serum the values were 38.8 ± 8.6 and 40.4 ± 10.6 for R- and S-propranolol, and 34.7 ± 10.5 and 44.8 ± 10.7 for R- and S-verapamil. For propranolol, in maternal, but not in foetal serum, the difference between the binding of the R- and S-enantiomers was significant; the R/S ratio was significantly ($P < 0.01$) larger in the mother (1.099 ± 0.072) than in the foetus (0.973 ± 0.068). For verapamil, the difference in binding between the R- and S-enantiomers was significant in both maternal and foetal serum, but the R/S ratio was similar in mother (0.735 ± 0.098) and foetus (0.763 ± 0.070). Serum α_1 -acid glycoprotein (AAG) concentrations were markedly higher and albumin concentrations slightly lower in maternal than in foetal samples. The binding of the four enantiomers in maternal and foetal serum was correlated ($P < 0.001$) with the AAG concentration (r propranolol: R 0.749, S 0.746; r verapamil: R 0.753, S 0.782). Our findings show that measurement of concentrations of total, unresolved drug allow a reasonably accurate assessment of transplacental gradients of individual isomers.

Keywords protein binding enantiomers propranolol verapamil maternal serum foetal serum α_1 -acid glycoprotein

Introduction

For drugs bound mainly to α_1 -acid glycoprotein (AAG), binding in foetal serum is lower than in maternal serum, an observation that has been explained by a lower foetal α_1 -acid glycoprotein (AAG) concentration [1, 2]. Many drugs are used as racemic mixtures, and differences in the serum binding of their enantiomers in adults have been reported [3]. It is not known to what extent binding in foetal serum is enantioselective. Therefore we have measured the binding of the isomers of propranolol and verapamil in pairs of maternal and foetal serum samples obtained at delivery; both drugs are bound to AAG and show enantioselective serum binding in adults [4–6]. We also determined whether, based on the foetal/maternal AAG concentration ratio, foetal binding of the enantiomers can be predicted from maternal binding data [1].

Methods

Materials

R(+)- and S(–)-propranolol were gifts from ICI (Macclesfield, UK) and R(+)- and S(–)-verapamil were gifts from Knoll (Ludwigshafen, Germany). [³H]-propranolol (specific activity 91 μ Ci mg^{-1} ; radiochemical purity > 99.0%) was purchased from Amersham International (Amersham, UK) and [³H]-verapamil (specific activity 220 μ Ci mg^{-1} ; radiochemical purity > 99.3%) from Dupont de Nemours (Brussels, Belgium). Preparative resolution of the labelled propranolol and verapamil enantiomers was performed by h.p.l.c. [7, 8].

Subjects and blood sampling

Approval for this study was granted by the Ethics Committee of the Gent Medical School. Blood samples

were obtained at delivery from 19 women (age 22 to 39 years) and their newborn infants (gestational age 27 to 40 weeks). Bupivacaine was given for epidural anaesthesia to 13 women. Other drugs administered during delivery were oxytocin (10 women) and ritodrine (three women); one woman was treated during pregnancy with pyrimethamine and sulphadiazine, one with pethidine and L-thyroxine, and one with ampicillin. Samples from the mothers were collected by venepuncture either just before delivery (six cases) or just before epidural anaesthesia with bupivacaine which was given 2 to 6 h before delivery. Foetal venous blood samples were obtained from the umbilical cord. The samples were collected in glass tubes and centrifuged at 1000 *g* for 10 min. Serum samples were stored at -20°C .

Protein binding

Drug binding in serum was determined by equilibrium dialysis at 37°C as described previously [8]. The phosphate buffer (0.15 M, pH 7.4) contained the labelled enantiomer (50 ng ml $^{-1}$, 0.25 $\mu\text{Ci ml}^{-1}$). Before dialysis, the serum was adjusted to pH 7.4 by addition of 0.1 N HCl. The coefficients of variation for replicate assays of the binding of R-propranolol and S-verapamil were 7.5% and 9.3%, respectively ($n = 17$).

Measurement of albumin and AAG

The concentration of AAG was determined by turbidimetry using rabbit anti-human orosomucoid antibodies (Dako, Glostrup, Denmark) and that of albumin by the bromocresol green method (Boehringer, Mannheim, Germany). A Hitachi 717 analyser was used. The coefficients of variation for replicate assays of AAG (86 mg%) and albumin (3.66 g%) were 4.28% and 1.39%, respectively ($n = 10$). The detection limit for the AAG assay was 2 mg%.

Data analysis

A binding ratio (B/F) was predicted for each foetal serum sample from the product of the maternal binding ratio and the foetal/maternal serum AAG concentration ratio [1]. The differences in binding between R- and S-enantiomers were evaluated using the Wilcoxon rank order test for paired data. The same test was used for comparisons of the mean values of the binding data and the serum protein concentrations in the foetus with those in the mother. Comparison of the mean values of the foetal binding data from mothers who received bupivacaine with those who did not, were made using the Mann-Whitney U-test. Correlations between the binding ratios (bound/free) and the protein concentrations, and between the predicted and the observed foetal binding values were assessed by the Spearman rank order correlation test. A probability of less than 0.05 was considered statistically significant.

Results

The mean free percentages of propranolol and verapamil enantiomers in maternal and foetal sera are shown in Table 1, together with the R/S ratios and the mean differences between mother and foetus. The free percentages of the four enantiomers were markedly lower in maternal than in foetal serum. For propranolol, maternal binding was slightly but significantly higher for the S-enantiomer than for its antipode; in foetal serum no significant difference between the binding of the isomers was found. The R/S ratio of the free propranolol percentages was significantly lower in foetal than in maternal serum. For verapamil, there was a difference in binding between the R- and S-enantiomers in both maternal and foetal serum; there was no significant difference in the R/S

Table 1 Free percentages of propranolol and verapamil enantiomers and their R/S ratios, and AAG and albumin concentrations in foetal and maternal serum

	Foetus	Mother	Mean difference foetus – mother (95% confidence intervals)
Propranolol			
% free R	38.8 $\pm$ 8.6	22.4 $\pm$ 6.2	16.3 $\ddagger$ (11.9 to 20.8)
% free S	40.4 $\pm$ 10.6	20.7 $\pm$ 6.6*	19.7 $\ddagger$ (14.2 to 25.1)
R/S	0.973 $\pm$ 0.068	1.099 $\pm$ 0.072	–0.126 $\ddagger$ (–0.169 to –0.082)
Verapamil			
% free R	34.7 $\pm$ 10.5	16.8 $\pm$ 5.5	17.9 $\ddagger$ (12.5 to 23.3)
% free S	44.8 $\pm$ 10.7*	22.5 $\pm$ 6.2*	22.3 $\ddagger$ (16.8 to 27.7)
R/S	0.763 $\pm$ 0.070	0.735 $\pm$ 0.098	0.028 (–0.024 to 0.080)
AAG (mg%)	14 $\pm$ 8	66 $\pm$ 24	–52 $\ddagger$ (–63 to –41)
Albumin (g%)	4.07 $\pm$ 0.30	3.72 $\pm$ 0.18	0.35 $\ddagger$ (0.20 to 0.50)

Means $\pm$ s.d. are shown ($n = 19$).

*, $P < 0.001$ S- vs R-enantiomer; $\ddagger$, $P < 0.01$, $\ddagger$, $P < 0.001$ maternal vs foetal values (Wilcoxon rank test for paired data).

ratios of the free verapamil percentages between mother and foetus. There was no significant difference in binding of the enantiomers of both drugs between the foetuses from mothers who received bupivacaine and those whose mothers did not.

Serum albumin concentrations were slightly higher ($P < 0.01$) and AAG concentrations markedly lower ($P < 0.001$) in foetal than in maternal serum; the mean foetal/maternal serum AAG concentration ratio was 0.23 (Table 1). There was a positive correlation between the binding ratios (bound/free) and the AAG concentrations for the propranolol enantiomers ($r = 0.749$ (R), $P < 0.001$; $r = 0.746$ (S), $P < 0.001$) and for the verapamil enantiomers ($r = 0.753$ (R) $P < 0.001$; $r = 0.782$ (S), $P < 0.001$). There was no significant correlation with the albumin concentrations. No statistically significant correlations between predicted and observed foetal binding data were found (Figure 1).

Discussion

The free percentages of both enantiomers of propranolol and verapamil were higher in foetal than in maternal serum. This is in agreement with findings for racemic propranolol [9]; for verapamil no data are available. A difference in binding protein concentrations is one of the factors responsible for the difference in binding between maternal and foetal serum [1]; for both drugs, significant correlations between the bound/free ratios of the enantiomers and the

serum AAG concentrations were found, suggesting that the lower binding of both drugs in neonatal serum is, indeed, related to the lower AAG levels. One woman delivered at 27 weeks: the premature neonate had the lowest AAG concentration and, for both drugs, the highest free fraction. It is known that serum AAG levels are lower in premature babies than in neonates born at term [10].

In maternal serum there was a small but significant difference in binding between the propranolol enantiomers, as reported previously in non-pregnant subjects [4, 6]; in foetal serum the difference in binding between the propranolol enantiomers was not statistically significant. For verapamil, the difference in binding between the enantiomers was slightly, but significantly greater in maternal than in foetal serum. It can be concluded from these results that, for the two drugs studied, the differences in serum binding between the R- and S-enantiomers are similar in mother and foetus. This finding is relevant to the interpretation of the transplacental passage of drugs. Thus, measurement of concentrations of total, unresolved drug should give a reasonably accurate assessment of transplacental gradients of individual isomers.

In contrast to the findings of Hill & Abramson [1] we found no correlation between predicted and observed foetal drug binding, suggesting that factors other than the binding protein concentration (e.g. differences in protein structure or the presence of competing ligands) are important.

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Figure 1 Predicted vs observed foetal serum binding ratios (B/F: bound over free) of R- and S-propranolol and R- and S-verapamil. Predicted values were calculated in each foetal sample from the product of the maternal binding ratio and the foetal/maternal AAG concentration ratio. Correlations were assessed by the Spearman rank order test.

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