Displacement of bilirubin from cord serum by sulphadimethoxine, amoxycillin, clavulanic acid in combination with either amoxycillin or ticarcillin, temocillin and cloxacillin

B. E. DAVIES

Drug Metabolism and Pharmacokinetics Department, Beecham Pharmaceuticals Research Division, Coldharbour Road, The Pinnacles, Harlow, Essex, UK

- 1 The displacement of bilirubin from its binding sites in cord serum by sulphadimethoxine and various penicillins was studied using Sephadex gel filtration.
- 2 Sulphadimethoxine displaced bilirubin at concentrations well within the normal therapeutic range of sulphonamide concentrations.
- 3 Amoxycillin, clavulanic acid in combination with either amoxycillin or ticarcillin, temocillin and cloxacillin displaced bilirubin at concentrations much higher than those found clinically.
- 4 It was concluded that the primary binding site/s of sulphadimethoxine and the secondary binding site/s of the penicillins were at or near the primary bilirubin binding site.

Keywords bilirubin displacement cord serum penicillin sulphonamide

Introduction

Unconjugated hyperbilirubinaemia is regularly observed during the first days of life and is particularly pronounced in prematurity, haemolytic disease, and severe infections. Human neonates conjugate bilirubin poorly during the first week of life due to the immaturity of hepatic glucuronyl transferase. Clinical experience has shown that treatment of icteric newborns with drugs that compete with bilirubin for its binding sites can cause displacement of bilirubin from blood albumin into the central nervous system, resulting in kernicterus. As antimicrobial agents, some of which such as cloxacillin are highly protein bound, are frequently administered during the first few days of life, an important objective of preclinical studies should be to study the interaction between antimicrobials and bilirubin. Previous studies on semi-synthetic penicillins reported that ampicillin at the concentrations studied, had no effect on bilirubin binding but that cloxacillin and flucloxacillin both displaced bilirubin from pooled cord serum (Friedman & Lewis, 1980).

The objective of the present study was to investigate the potential of amoxycillin, clavulanic acid in combination with either amoxycillin or ticarcillin (Augmentin and Timentin, Beecham plc respectively), cloxacillin and temocillin (BRL 17421) to displace bilirubin from human cord serum using an *in vitro* method of measuring bilirubin binding. Sulphadimethoxine was also studied because it has been shown to be an effective displacer of bilirubin *in vitro* (Yeary *et al.*, 1977).

Methods

Blood from neonates was collected from the maternal end of the umbilical cord immediately after birth. Serum was obtained by centrifugation and stored at -20°C before use. Bilirubin binding was studied using a method based on that of Chunga & Lardinois (1971). Glass columns (10 cm $\times$ 1.0 cm), wrapped in aluminium foil, containing 500 mg of Sephadex G-25 beads

Correspondence: Mr B. E. Davies, Drug Metabolism and Pharmacokinetics Department, Beecham Pharmaceuticals Research Division, Coldharbour Road, The Pinnacles, Harlow, Essex

(Pharmacia) were prepared with 0.1 M phosphate buffer pH 7.4. Anhydrous bilirubin (50 mg, Sigma) was dissolved in a minimum amount of 0.1 N NaOH and the volume made up to 10 ml with 0.1 M phosphate buffer pH 7.4. Aliquots (10 μ l) of this solution were immediately added to samples of 0.5 ml human cord serum to which had been added sulphadimethoxine (Sigma), amoxycillin, clavulanic acid combined with ticarcillin, clavulanic acid combined with amoxycillin, cloxacillin or temocillin dissolved in pH 7.4 phosphate buffer over the concentration range 0–1000 μ g ml⁻¹. The cord serum sample (0.5 ml) was immediately placed on top of the Sephadex column and protein bound bilirubin was eluted with 6 ml of pH 7.4 phosphate buffer. Free bilirubin was eluted with 7.5 ml of 0.1 N NaOH into 2 ml of a solution of 0.1 M ascorbic acid and 0.5 M citric acid and extracted into 1 ml of chloroform. The absorbance of free bilirubin in the chloroform extract was determined at 450 nm. The lowest drug concentration at which measurable drug-bilirubin interaction would be expected to occur, the threshold concentration, was calculated by extrapolation of the logarithm of drug concentration vs percent unbound bilirubin regression line to zero effect. The apparent dissociation constant of drug from the bilirubin binding site (K_d) and maximum percent free bilirubin (E_{max}) were calculated from a double reciprocal plot according to the equation:

$$1/\% \text{ unbound bilirubin} = \frac{1}{E_{max}} + \frac{K_d}{E_{max}} \cdot \frac{1}{\text{Drug concentration}}$$

Results

The percentage free bilirubin values obtained with increasing drug concentration are pre-

Figure 1 Displacement of bilirubin from cord serum by increasing concentrations of sulphadimethoxine and various penicillins, ● sulphadimethoxine, ■ cloxacillin, ○ BRL 17421, ▲ clavulanic acid/amoxycillin, □ clavulanic acid/ticarcillin, ▽ amoxycillin.

sented graphically in Figure 1. All of the drugs displaced bilirubin to some extent with sulphadimethoxine by far the most effective displacer. Figure 2 is the sulphadimethoxine double reciprocal plot and from this, values for K_d and E_{max} of 3.34 mM and 9.21% were obtained. From a plot of log drug concentration vs percent unbound bilirubin a threshold concentration for sulphadimethoxine of 55.9 μ g ml⁻¹ was obtained. These values are presented in Table 1. As there was only minimal displacement of bilirubin by the antibiotics, very little data were obtained to characterise the displacement process. Even so, K_d and E_{max} values were calculated and also presented in Table 1. Threshold concentrations ranged from 325 to 385 μ g ml⁻¹ (0.78 to 1.29 mM) with a mean value of 358 μ g ml⁻¹ (0.98 mM). E_{max} values for the antibiotics ranged from 0.24 to 0.91% with a mean value of 0.54% and K_d values ranged from 0.64 to 1.74 mM with a mean value of 1.04 mM.

Discussion

Sulphadimethoxine was the most effective displacer of bilirubin from cord serum with a threshold concentration of 55.9 μ g ml⁻¹ and K_d and E_{max} values of 3.3 mM and 9.21%, respectively. These results compare favourably to those of Yeary *et al.* (1977) who obtained values of 131 μ g ml⁻¹, 3.13 mM and 8.2% for the threshold concentration, K_d and E_{max} , respectively, using adult human serum. The threshold concentration of 55.9 μ g ml⁻¹ (0.18 mM) is well within the therapeutic range of sulphonamides which are normally present at a peak concentration of 0.5 to 1.0 mM (Yeary & Davis, 1974; Bratlid & Bergan, 1976).

None of the antibiotics displaced bilirubin until quite high concentrations were achieved, with threshold concentrations in the range of

Figure 2 Double reciprocal plot of bilirubin displacement from cord serum by increasing concentrations of sulphadimethoxine.

Table 1 Maximum displacement, dissociation constants and threshold concentrations for sulphadimethoxine and various antibiotics

Drug	$E_{\max}$ (%)	K_d (mM)	Threshold concentration	
			(mM)	($\mu\text{g ml}^{-1}$)
Sulphadimethoxine	9.21	3.34	0.18	55.9
Cloxacillin	0.91	1.26	0.78	340
Temocillin (BRL 17421)	0.46	0.68	0.89	370
Clavulanic acid in combination with amoxycillin*	0.24	0.64	1.29	369
Clavulanic acid in combination with ticarcillin**	0.51	0.87	1.05	385
Amoxycillin	0.56	1.74	0.89	325

* 2 : 1 mixture of amoxycillin and clavulanic acid

** 20 : 1 mixture of ticarcillin and clavulanic acid

0.78 to 1.29 mM. These threshold concentrations are more than 10 fold higher than the oral therapeutic concentration of penicillins which are usually present at around 0.05 mM (Cohen *et al.*, 1975; Bratlid & Bergan, 1976; Friedman & Lewis, 1977). For those penicillins administered by the intravenous route, the maximum attained plasma or serum concentration during therapy ranged from 0.3 to 0.35 mM (Brogden *et al.*, 1980). The threshold concentrations of temocillin and ticarcillin in combination with clavulanic acid were at least 2.5-fold greater than the therapeutic concentrations. Following oral administration of clavulanic acid combined with amoxycillin (Jackson *et al.*, 1980) and following intravenous administration of clavulanic acid with ticarcillin (Staniforth *et al.*, 1985) the areas under the plasma concentration-time curves of clavulanic acid and either amoxycillin or ticarcillin were in the same ratio as in the original dose formulation. Therefore, the ratios of clavulanic acid with either amoxycillin or ticarcillin used for this *in vitro* study would be the same as the ratio of plasma concentrations obtained *in vivo* following administration of the two combinations. The values of K_d and $E_{\max}$ for the antibiotics were much lower than those of sulphadimethoxine reflecting their lower potential for displacement of bilirubin. Even at the higher antibiotic concentrations the percentage free bilirubin was only increased two-fold. The extent of protein binding of the majority of the antibiotics studied is relatively low except for cloxacillin which is 94% bound (Rolinson & Sutherland, 1965). The threshold concentration of cloxacillin is lower and $E_{\max}$ and K_d values higher than those of the majority of the other antibiotics studied which agrees favourably with the data of Friedman & Lewis (1980), who indi-

cated that cloxacillin and flucloxacillin had a greater potential than ampicillin to displace bilirubin.

Kazuo *et al.* (1984) have classified certain drugs based on their ability to displace bilirubin from its primary binding site on human serum albumin. Group 1 drugs at a molar ratio of drug to albumin of < 1.5 : 1 effectively displaced bilirubin suggesting that the area containing their primary binding sites overlapped the bilirubin site. Group 2 drugs at concentrations up to a molar ratio of 1.5 did not change free bilirubin concentration. However, at about two-fold molar excess of these drugs over albumin, bilirubin was significantly displaced. From the results of the present study, sulphadimethoxine (molar ratio of 0.32) can be considered as a Group 1 drug, based on the classification of Kazuo *et al.* (1984). Displacement of bilirubin by the antibiotics, in the present study, occurred at a molar ratio of 1.5 to 2 suggesting that the primary binding site of the antibiotics does not overlap the bilirubin site, but that the secondary binding site does. From these observations, the antibiotics can be considered as Group 2 drugs.

In the light of the above considerations, it would seem unlikely that amoxycillin, cloxacillin, clavulanic acid in combination with either ticarcillin or amoxycillin and temocillin, unlike the sulphonamides, would lead to displacement of bilirubin from albumin when administered to jaundiced human neonates.

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