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THE EFFECT OF DRUGS ON BILIRUBIN-ALBUMIN BINDING CAPACITY

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INDEXING WORDS

bilirubin-albumin binding; displacing effect; antimicrobial drugs; hyperbilirubinemia; kernicterus; unbound bilirubin; neonate

SYNOPSIS

Displacement of bilirubin-bound to human serum albumin by 14 commonly used drugs were studied quantitatively using a model of competitive binding of bilirubin and drug to one site on serum albumin. The rate of bilirubin displacement by certain concentrations of drugs were measured by peroxidase oxidation method. The clinically potent bilirubin-displacer, sulfisoxazole was applied as control.

A linear plot of relative increase of the unbound bilirubin levels after addition of certain levels of drugs were obtained. The constant binding of sulfisoxazole was $1.72 \times 10^{4} M^{-1}$ and the maximal displacing factor (MDF) in treated babies was estimated at 2.29. Taking the MDF 1.2 as the upper limit for significant danger

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of displacement, latamoxef (LMOX - 1.63) belonged to this group. Cefalotin (CET - 1.13) had a moderate displacing effect and slight effects were shown by cefotaxime (CTX - 1.07), acetyl salicylic acid (1.08), aminophylline (1.06). No displacing effects were recorded on furosemide, diazepam, phenobarbital, doxapram, digoxin, ampicillin, gentamicin, amikacin and kanamycin.

It is concluded that caution should be taken in giving latamoxef to jaundiced infants with infection for the increasing risk of kernicterus.

INTRODUCTION

Some drugs given to the newborn infants are able to displace bilirubin from albumin, resulting in increased concentration of unbound bilirubin and increasing the risk of kernicterus.[1)] Clinical experience has shown that treatment of icteric premature infants with sulfisoxazole (gantrisin) can cause fatal kernicterus.[12)]

Stern[13)] stressed that any drugs used in the newborn infant should be examined for its ability to displace bilirubin from albumin. Even during pregnancy, a displacing drug may reach the fetus via the placenta and exert its effect on fetal hyperbilirubinemia. The peroxidase method measures the rate of oxidation of bilirubin in mixtures of albumin in the presence of hydrogen peroxide and peroxidase. This method can be used as a screening test for displacement of bilirubin by drugs.[1, 4)]

In this report, we evaluate the displacement effect of some antimicrobials and other commonly used drugs in the newborn infants in vitro. Sulfisoxazole was used as control.

MATERIALS AND METHODS

The drugs for in vitro studies were obtained commercially. The drugs were:

- antimicrobials: ampicillin, gentamicin, amikacin, kanamycin, cefalotin, cefotaxime, latamoxef;
- others: furosemide, diazepam, phenobarbital, digoxin, aminophylline, doxapram, acetyl salicylic acid.

The powder form of drugs were dissolved in distilled water.

In case of low solubility (furosemide, phenobarbital, digoxin, doxapram) NaOH 0.1 N solution was used. Sulfisoxazole 10% solution for injection was performed as control.

Human serum albumin powder and crystalline bilirubin were obtained from Sigma Chemical Co. (St. Louis). Standard bilirubin-albumin solution was made by mixture of albumin dissolved in phosphate buffer and bilirubin plus small volume of NaOH 0.1 N in distilled water. it contained of 0.255 mM bilirubin, 0.45 mM albumin, and unbound bilirubin 0.022 μM. The bilirubin-albumin standard was freshly made for testing of the drugs.

Varying concentrations of drug were added to standard solution and the oxidation rate of unbound bilirubin was measured by using UB-analyzer.[11] The reaction was conducted in pH 7.4, 30C. Every drug and its certain final concentration were examined in triplicates. The coefficient variation of analysis was less than 5%. As far as possible the range of the final concentrations of the drugs covered the therapeutic blood levels. None of the drugs studied inhibited the peroxidase process in the absence of albumin. All procedures were performed in subdued illumination and cuvettes were protected by aluminium foil.

The result of the test can be evaluated in term of maximal displacing factor (MDF):

$MDF = Kd \cdot d + 1 = Kd \cdot (1-q) \cdot D + 1$; where

Kd (binding constant of the drug) is determined as a slope of the oxidation rate, relative to the rate in the absence of drug, plotted as a function of added drug concentration. This is a factor for assessing the effect of drug on a competition between one molecule of bilirubin and one molecule of drug for a specific site on the albumin molecule. d is the free drug concentration needed for therapeutic effect, consisting of the total plasma concentration (D) and degree of protein binding of the drug (q). The MDF denotes a quantitative index of the risk of bilirubin displacement incurredly the use of a drug at a certain free concentration in the plasma. An upper limit of MDF of 1.2 has been proposed for significant displacement, which corresponds to a decrease in the reserve albumin of 17%.[4, 5]

RESULTS

As shown in Figure 1 the binding constant of diazepam and furosemide were higher than that of sulfisoxazole. Further calculations of MDF were needed for clarification the displacing effect. No slope of oxidation rate was found on ampicillin, gentamicin, amikacin, kanamycin, digoxin and doxapram. These drugs could be taken as nondisplacing.

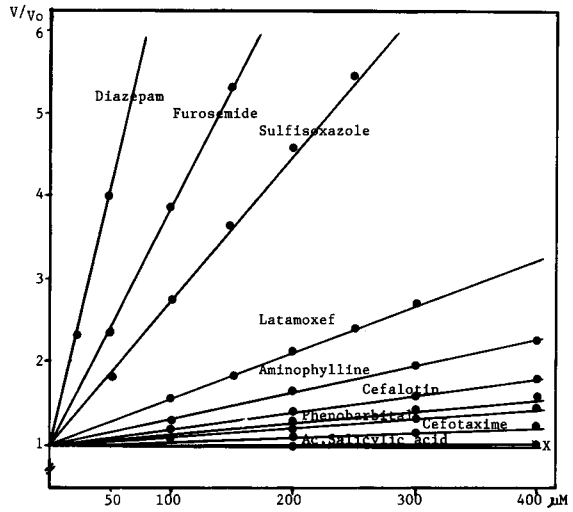

Fig. 1 Increase in rates of bilirubin oxidation with peroxidase (bilirubin: albumin molar ratio 0.56) by sulfisoxazole and 14 drugs tested. Ordinate V/Vo, the relative increases in oxidation rates of bilirubin compared to control with no drug added. Absciss, the final concentrations of drugs.

The calculation of binding constant and MDF of the drugs are shown in Table 1. Sulfisoxazole as clinically known bilirubin displacer had Kd of $1.72 \times 10^{4} M^{-1}$ and MDF 2.29. Taking the MDF 1.2 as the upper limit of significant displacer, latamoxef can be considered as a displacer drug. Cefalotin has a moderate displacing effect and slight effects are suspected on cefotaxime, acetyl salicylic acid and aminophylline.

Table 1 Binding constant (Kd) and maximal displacing factor (MDF) of the drugs studied (pH 7.4, 30C, 0.1 M phosphate buffer).

	Kd (M-1)	Protein binding (%)	Plasma concentration (M)	MDF
Diazepam	6.7×10^{4}	98.7	0.1×10^{-5}	1.0009
Furosemide	3×10^{4}	98.8	1.82×10^{-5}	1.006
Sulfisoxazole	1.72×10^{4}	85	0.5×10^{-3}	2.29 *
Latamoxef	5.3×10^{3}	50	2.37×10^{-4}	1.628*
Aminophylline	3.1×10^{3}	56	4.38×10^{-5}	1.059
Cefalotin	2.1×10^{3}	70	1.79×10^{-4}	1.113°
Phenobarbital	7.18×10^{2}	50	1×10^{-4}	1.036
Cefotaxime	7.14×10^{2}	36	1.5×10^{-4}	1.068
Ac. Salicylic acid	3.2×10^{2}	84	5×10^{-4}	1.08
Digoxin	1.1×10^{2}	25	1.5×10^{-9}	1
Doxapram	-	50	2.31×10^{-5}	1
Ampicillin	1.4×10^{2}	18	1.34×10^{-4}	1.015
Amikacin	1.3×10^{2}	4	0.1×10^{-4}	1
Kanamycin	-	0	0.5×10^{-4}	1
Gentamicin	-	0	0.6×10^{-4}	1

* Significant displacer, ° moderate displacer

DISCUSSION

Previous works found that gentamicin interfered with bilirubin-albumin binding in vitro and on animal experiments.[7, 13] Other studies reported that it belonged to non displacer drug.[1, 8, 15] The preservative in the drug preparation was thought to be the interfering agent, as methylparaben was a potent displacer of bilirubin from its secondary binding sites and a weak competitor with bilirubin for binding to primary binding sites.[9] Recently on quantitative in vivo and in vitro studies, Brodersen and Ebbesen[3] did not find any measurable displacement of bilirubin by gentamicin. They also found ampicillin had a slight displacing effect.

Among cephalosphorine derivates, latamoxef had a significant bilirubin displacement ability. Stutman et al.[14] reported that moxalactam displaced bilirubin from albumin when the concentration was 18 μM (130 μg/ml) or higher. The average peak plasma concentrations were 123 and 234 μg/ml after moxalactam intravenous administration of 50 and 100 mg/kg, respectively.

According to one report, furosemide, like sulfisoxazole was a potent displacer of bilirubin and should be used with caution in jaundiced infants in the first few days of life.[10] However, Cashore et al.[6] concluded that peak plasma levels acheivable with 1 mg/kg of furosemide were not high enough to produce significant reduction of reserve albumin. This was in accordance with our result that although the binding constant of furosemide was higher than sulfisoxazole, the MDF showed insignificant figure. The same phenomenon happened on diazepam which was also categorized as non displacer drug.

Aminophylline was found to be a weak bilirubin displacer, which was in accord with another report.[2] Other drug used for apnea in prematurity, doxapram was non displacer.

The clinical significance of these data implicate latamoxef as a risk factor in jaundiced neonates and caution is suggested in the therapeutic use. The moderate and slight displacing drugs will take account as risk factor in exessive blood level and low serum reserve albumin binding capacity.

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