

## ORIGINAL ARTICLE

## Effect of Cloxacillin on Bilirubin-albumin Binding

by

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### Abstract

*Cloxacillin is usually given to newborn infants with sepsis in particular for penicillinase-producing staphylococcus aureus. The effect of cloxacillin on bilirubin-albumin binding was investigated in vitro using the peroxidase oxidation method with human albumin (bilirubin 0.255 mM, albumin 0.45 mM, bil/alb 0.56, pH 7.4, temperature 30°C). Sulfisoxazole, a drug which is capable of displacing bilirubin from albumin was used as control.*

*The displacement constant of cloxacillin was  $4.86 \times 10^5 M^{-1}$ , stronger than that of sulfisoxazole ( $1.72 \times 10^4 M^{-1}$ ). Both drugs were capable of displacing bilirubin as determined by their maximal displacement factors of 2.21 and 2.29, respectively. Since cloxacillin apparently increases the risk of bilirubin encephalopathy the use of this drug in jaundiced newborns with sepsis, especially in the premature infants, should be reconsidered.*

## Introduction

Any drug that competes with bilirubin for its binding to albumin can increase the serum unbound bilirubin and may increase the risk of bilirubin encephalopathy in newborn infants with jaundice [1,2]. Antibiotics are commonly used in infants admitted to the neonatal intensive care units, having more risk of hyperbilirubinemia due to prematurity, asphyxia, infections or

other deteriorating conditions. The displacing effects of some antibiotic agents on the bilirubin - albumin binding have been reported in the previous study [3].

The aim of this study is to examine the displacement effect of cloxacillin on bilirubin - albumin binding *in vitro*. Sulfisoxazole, a known clinically potent bilirubin-displacer was used as control.

## Materials and Methods

Standard bilirubin - albumin binding solution was made by a mixture of albumin dissolved in phosphate buffer and bilirubin plus a small volume of NaOH 0.1 N distilled water. It contained 0.255 mM bilirubin, 0.45 mM albumin, and unbound bilirubin 0.022  $\mu$ M. This standard solution was freshly made for every testing. Human serum albumin powder and crystalline bilirubin were obtained from Sigma Chemical Co. (St. Louis).

Cloxacillin was obtained commercially. The powder form was dissolved in distilled water for a certain concentration (0.5 mM). Sulfisoxazole 10% solution for injection was used as control. Varying concentrations of drugs were added to the standard solution and the oxydation rate of the unbound bilirubin was measured by using UB-analyzer [4]. The reaction was conducted in pH 7.4, temperature 30°C. Cloxacillin and sulfisoxazole and its certain final concentration were examined in triplicates. The coefficient variation of analysis was less than 5% at the ranges of concentration measured. The range of final concentrations of both drugs covered the therapeutic blood levels. None of both drugs inhibited the peroxidase oxydation pro-

cess in the absence of albumin. All procedures were performed in subdued illumination and cuvetts were protected by aluminium foil. The result of the test can be evaluated in term of maximal displacing factor (MDF) [5]:

$$\text{MDF} = K_d \cdot d + 1 = k_d \cdot (1-q) \cdot D + 1$$
; where  $K_d$  (binding constant of the drug) is determined from the slope of the oxydation rate, relative to the rate in the absence of the drug, plotted as a function of added drug concentration. This is a factor for assessing the effect of drug in the competition between one molecule of bilirubin and one molecule of drug for a specific binding site in 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 incurred by 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% [5].

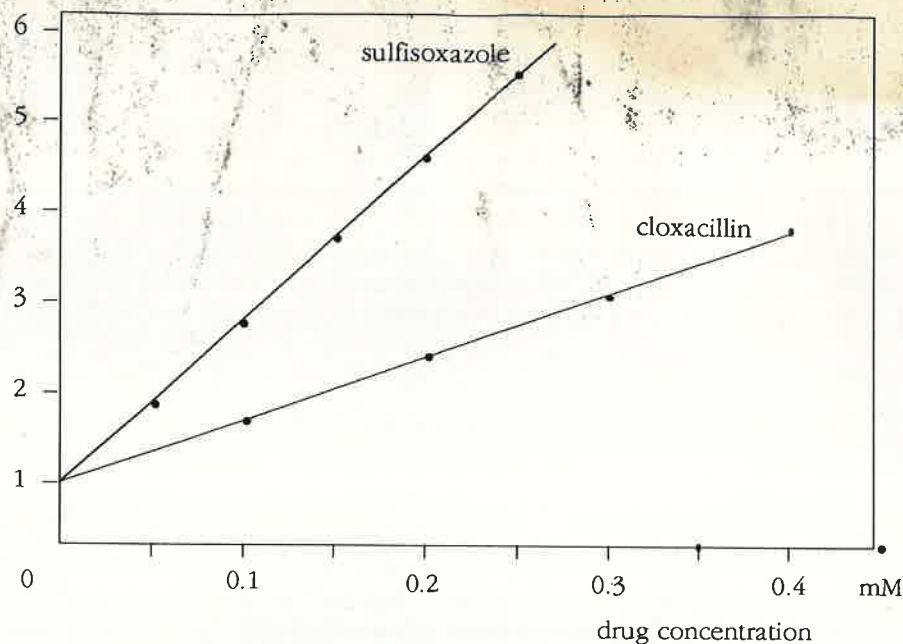


Figure 1. Rate of bilirubin displacement by sulfisoxazole and cloxacillin. Ordinate  $V/V_o$ , the relative increases in oxidation rates of bilirubin compared to standard with no drug added

Table I.  $K_d$  and MDF of sulfisoxazole and cloxacillin (pH 7.4, 0.1 M P04 buffer, 30°C)

	$K_d$ ( $m^{-1}$ )	Protein Binding (%)	Plasma Level (M)	MDF
Sulfisoxazole	$1.72 \times 10^4$	85	$0.5 \times 10^{-3}$	2.29
Cloxacillin	$4.86 \times 10^5$	95	$0.5 \times 10^{-4}$	2.215

## Results

The increase in reaction velocity indicates the competition between drugs (sulfisoxazole, cloxacillin) and bilirubin for albumin binding (Figure 1). The calculation of binding constant and MDF of the drugs are shown in Table I. Sulfisoxazole had  $K_d$  of  $1.72 \times 10^4 M^{-1}$  and MDF 2.29. Whereas cloxacillin had  $K_d$  of  $4.86 \times 10^5 M^{-1}$  and MDF 2.215. Taking the MDF 1.2 as the upper limit of significant displacer, cloxacillin could be considered as a strong displacer drug.

## Discussion

This study demonstrated that cloxacillin had a strong effect on the bilirubin-albumin binding in vitro. This drug has been indicated for the treatment of staphylococcal infections, in particular those caused by penicillinase-producing staphylococci.

No slope of oxydation rate was found on other antibiotics tested previously i.e. ampicillin, gentamicin, amikacin, kanamycin. Slight to moderate displacing effect were found for cefalotin, cefotaxime and latamofex [3]. Fink et al. (1988) [6] stud-

ied various penicillins available in the United States for the effects on bilirubin-albumin binding using similar techniques. Cloxacillin was not included. They found slight effects of some penicillins on bilirubin-albumin binding.

It is suggested to consider the use of cloxacillin in jaundiced infants with sepsis, especially in premature infants because of its strong displacing bilirubin effect. It is also stressed that any drug used in the newborn infants should be examined for its ability to displace bilirubin from albumin.

## REFERENCES

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