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Distribution of ampicillin in human whole blood

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Ampicillin is an antibiotic with a low degree of plasma protein binding (Ehrnebo, Agurell & others, 1971). It has previously been shown by several authors that many drugs are bound to the human red blood cells (cf. Ehrnebo, Agurell & others 1974; Hinderling, Bres & Garrett, 1974; Ehrnebo & Odar-Cederlöf, 1977). Suggested mechanisms are binding to intracellular haemoglobin (Wind, Berliner & Stern, 1973), carbonic anhydrase (Beerman, Hellström & others, 1975) and the erythrocyte membrane (Kwant & Seeman, 1969). However, few data are available in the literature on the distribution of ampicillin in human whole blood. In the following study, the equilibrium dialysis technique was used to study the distribution of [^{35}S]ampicillin to the blood cells, plasma proteins and plasma water of human whole blood.

Heparinized whole blood was drawn from five healthy male volunteers, aged 23–28 years. Human plasma, whole blood or a suspension of washed blood cells (500 μl) was introduced on one side of the dialysis membrane (Visking dialysis tubing) in cells of lucite (Ehrnebo & others, 1971, 1974). On the other side was applied [^{35}S]ampicillin (radiochemical purity >98%) in isotonic phosphate buffer pH 7·4. The cell was equilibrated for 5 h at 37°. There was no significant breakdown of ampicillin under these conditions as measured by microbiological assay. Samples (100 μl) were withdrawn from each side and concentration of drug in plasma (C_P), whole blood (C_B), blood cell suspension (C_{BCS}) and buffer solution (C_{BU}) was determined by liquid scintillation counting (Packard Tri-Carb model 3320) by means of the external standard channel ratio method. Samples containing red blood cells were digested with Soluene-100-isoproprenol (1:1) (Packard Instruments) and 30% hydrogen peroxide before adding the scintillation liquid (Instagel, Packard Instruments). The recovery of radioactivity in the digestion procedure was 96·7 s.d. 1·4% (n = 4) and correction for the loss of radioactivity was made before the calculations. The suspension of washed blood cells was prepared by washing the red cell fraction five times with isotonic phosphate buffer and reconstitution to the original haematocrit (McArthur, Dawkins & Smith, 1971). The fraction bound in plasma (f_P) was calculated as

$$f_P = (C_P - C_{BU})/C_P \quad (1)$$

The fraction distributed to the blood cells (λ_{BC}), plasma proteins (λ_{PP}) and plasma water (λ_{PW}) of the whole blood and blood cells (λ_{BCS}) of blood cell suspension was determined according to Ehrnebo & others (1974) as

$$\lambda_{BC} = 1 - C_{PW}(1\text{-}H)/(1\text{-}f_P)C_B \quad (2)$$

$$\lambda_{PP} = C_{PW} f_P (1\text{-}H)/(1\text{-}f_P)C_B \quad (3)$$

$$\lambda_{PW} = C_{PW}(1\text{-}H)/C_B \quad (4)$$

$$\lambda_{BCS} = 1 - C_{BU}(1-H)/C_{BCS} \quad .. \quad .. \quad (5)$$

where H is the haematocrit (fraction volume blood cells per volume blood).

As seen in Table 1, ampicillin had a low degree of plasma protein binding (f_P); the mean value of per cent bound in plasma was 17·8 s.d. 2·0%, which is in accordance with previous published values (Ehrnebo & others, 1971; Brusch, Bergeron & others, 1974). With reference to whole blood, this represents 17·5 s.d. 2·5% of the total amount in whole blood being distributed to the plasma proteins (Table 1).

Consequently, there was apparently no binding or distribution of ampicillin to the red blood cells of the whole blood, the percentage bound was 1·6 s.d. 3·8%, which was not significantly different from zero according to the two tailed Student's *t*-test (Table 1). When the erythrocytes were washed with buffer, it was possible to detect a slight binding of ampicillin to the cells. This represented only 6·1 s.d. 2·5% of total ampicillin in the blood cell suspension bound to the cells, the value was statistically greater than zero at the $P < 0.001$ level (two tailed Students' *t*-test). The mean value 6·1% is equal to a concentration ratio C_{cell}/C_{water} of only 0·08 for the washed blood cells. This slightly increased binding might be due to removal of some factor bound to the cell membrane, resulting in changed binding properties in favour of ampicillin.

There seems to be limited information in the literature concerning binding of ampicillin and other penicillins to human red blood cells. Nishida, Matsubara & others (1970) reported values for the binding of ampicillin to human erythrocytes and plasma obtained by means of microbiological assay. They suggested 4% incorporation into erythrocytes in suspension, 3% into erythrocyte of whole blood and only 3% bound in plasma, the last value is in contrast to the present report (18%) as well as previously published data (Ehrnebo & others, 1971; Brusch & others, 1974). Watson (1958) detected by a microbiological incubation technique, traces (10%) of penicillin G in washed erythrocytes. About the same value (5–6%) was found for [^{14}C]penicillin G bound to a washed erythrocyte suspension with 18% haematocrit by Kornguth & Kunin (1976). These authors also reported a 21–23% binding of dicloxacillin under the same conditions.

Table 1. *Distribution of ampicillin in human whole blood** in vitro.

Subject	H†	f_P	Fraction distributed to/in λ_{PW}	λ_{PP}	λ_{BC}	λ_{BCS}
I	0·43	0·161	0·826	0·158	0·016	0·025
II	0·42	0·199	0·836	0·207	−0·044	0·051
III	0·43	0·161	0·796	0·153	0·051	0·065
IV	0·48	0·168	0·795	0·160	0·046	0·075
V	0·47	0·201	0·790	0·198	0·012	0·089
Mean	0·45	0·178	0·809	0·175	0·016‡	0·061§
s.d.	0·03	0·020	0·021	0·025	0·038	0·025

* Total concentration approx. 50 μg ml^{-1}.
† Corrected for trapped plasma.
‡ Not statistically different from zero (Student's *t*-test, two tailed; $t = 0.942$; $2P > 0.3$).
§ Statistically greater than zero (Student's *t*-test, two tailed; $t = 5.456$; $2P < 0.001$).
H = haematocrit; f_P = fraction bound in plasma; λ_{PW} = fraction in plasma water; λ_{PP} = fraction in plasma proteins; λ_{BC} = fraction in red blood cells; λ_{BCS} = fraction to/in washed red blood cells.

In conclusion: There seems to be no significant binding to or distribution into the red blood cells of ampicillin in whole blood of healthy subjects. Only when the cells had been washed free of plasma, traces of binding could be detected.

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