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Binding of 3-Carbethoxipsoralen to Human Serum Albumin and Human Serum: Influence of Free Fatty Acids

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Characteristics of the binding of 3-carbethoxipsoralen (3CPS) to human serum albumin (HSA) and serum proteins have been studied. An electrophoretic study showed that the predominant binding protein fraction was albumin, with small binding to globulins. Binding to HSA, studied by equilibrium dialysis, is 75% and characterized by a small saturable number of binding sites (N = 0.27) with a moderate affinity constant ($K = 8 \times 10^4 M^{-1}$). Free fatty acids were shown to decrease 3CPS binding to HSA by a non competitive process.

Key words: 3-Carbethoxipsoralen, Serum binding, Albumin binding.

Psoralens are heterocyclic oxygenated coumpounds which are derivated from the furocoumarinic moiety. The psoralen derivatives are the following: natural occuring 5-methoxy or 8-methoxypsoralen (5MOP and 8MOP) (12), hemisynthetic 4,5',8-trimethylpsoralen (TMP) (8), 5'-aminomenthyl-trimenthylpsoralen (5'AMT) (6), or synthetic 3-carbethoxypsoralen (3CPS) (14). All these molecules are highly effective antipsoriatic drugs when associated with UV-A (PUVA-therapy) (5). 3CPS, the structure of which is shown on figure 1, is a new potential antipsoriatic agent now in clinical trial in France.

Chemical interactions between 3CPS and DNA or structural cutaneous proteins as keratins have been largely demonstrated (2, 10). A knowledge, therefore, of the intensity and mechanism of serum protein binding, particularly binding to human serum albumin (HSA), seems very important to understand the pharmacological behavior of 3CPS. Binding to HSA and total serum is presented in this report: the main serum carrier of 3CPS was assessed by a preliminary electrophoretic study; binding to HSA and serum was studied by equilib-

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rium dialysis. Furthermore, the effect of free fatty acids (FFA) on binding sites of 3CPS was estimated.

Materials and Methods

Electrophoresis. — The binding of 3CPS (Institut Curie, Orsay, France) on the different serum protein fractions was estimated by cellulose acetate electrophoresis performed at pH = 8.2 (barbital buffer). 3CPS was measured on total electrophoretical strip and on each protein fraction by high performance liquid chromatography (HPLC) with a fluorimetric detection (9), as described below.

Equilibrium dialysis. — 3CPS binding to HSA was studied by equilibrium dialysis, carried out at 37°C, pH = 7.4 (phosphate buffer, M/15) for 4 hours, under constant stirring at 20 rev/min (Dianorm* system). No significant binding occurred on dialysis membrane (Diachema*) or cell walls. At equilibrium, 3CPS concentrations were measured in each compartment by HPLC.

For the determination of the number of binding sites (N) and the affinity constant (K), 3CPS was used over a wide range of concentrations (0.194-193.8 μM or 0.05-50 μg/ml), recovering therapeutic levels (0.5-5 μg/ml); an albumin concentration of 2 g/l (29 μM) was used to reach a more rapid saturation of binding sites, as previously shown (14). The influence of FFA was assessed by binding of 3CPS to crude and defatted HSA (Sigma); the interaction was confirmed by binding to defatted HSA spiked with 50 μM FFA (expressed in stearic acid).

Binding of 3CPS to human serum was measured in physiological conditions, with an albumin concentration adjusted to 40 g/l, and was then compared with binding to crude and defatted HSA at 40 g/l. These experiments were performed with concentrations 0.1, 0.5 and 1 μg/ml of 3CPS.

3CPS determination. — 3CPS was measured by a fluorimetric HPLC method according to PROGNON *et al.* (9): briefly, the buffered solution of 3CPS spiked with ethanolic solution of TMP (Sigma) (internal standard) was extracted with 1 ml of methylene chloride; the organic layer was transferred and evaporated under gentle stream of nitrogen. The residue was dissolved in 50 μl of methanol, and an aliquot (20 μl) was injected into a Spherisorb 10 μm column (Waters).

Data analysis. — The binding parameters were roughly estimated according to SCATCHARD (13) then accurately calculated from the general formula derived from the mass law action, using a non linear least-squares regression (14, 17); the bound fraction (B) was plotted against the free fraction (F) of the drug; the number of binding sites (N) and the affinity constant (K) were performed on a computer Tektronik 4051 (14).

Results and Discussion

ELECTROPHORESIS

The binding of 3CPS to the different human serum protein fractions, (table I) shows a predominant binding of 3CPS to albumin (85%) and a slight binding to the globulins (15%). This value appears to be in close agreement with literature data for other psoralens (1, 3, 15, 16). However, repartition data of 3CPS is only a semi-quantitative indication of the main bind-

Table I. *3CPS binding to serum protein fractions.*

Proteins		3CPS binding (%)
Albumin		85.0
Globulins	α	4,8
	β	10.0
	γ	0.2

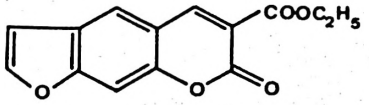

Fig. 1. *Structural formula of 3-Carbethoxipsoralen.*

ing proteins and must be carefully interpreted: the alcaline pH of barbital buffer implies a partial hydrolysis of the pyron ring of the furocoumarinic structure (4), and leads to a polar compound structurally related to cinnamic acid. At pH = 8.2, about 20% of 3CPS is in an «open ring» form. Therefore, the distribution of 3CPS on the protein fractions could be relatively different from its distribution at pH = 7.4. In spite of this, HSA remains the main carrier of 3CPS in the serum.

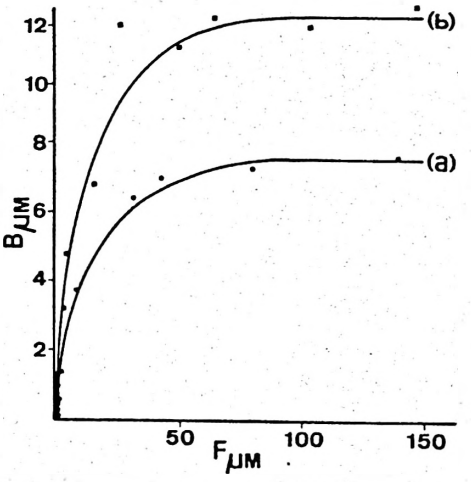

Fig. 2. *Binding of 3CPS (0.194-194 μM) to crude (a) or defatted (b) HSA (2 g/l or 29 μM).*
B and F are respectively bound and free concentrations. Each result is the mean of three determinations.

EQUILIBRIUM DIALYSIS

Binding of 3CPS to HSA: determination of binding parameters. — The binding percentage of 3CPS to HSA decreased when 3CPS concentration raised from 0.005 to 50 μg/ml: figure 2 shows a saturation of 3CPS binding sites to HSA. This behavior is characteristic of a saturable process with a limited number of binding sites (N = 0.275), as found for other psoralens (16); the moderate affinity constant (K = 8.8×10^4 M^{-1}) is in good agreement with the constant published for other psoralens.

Nevertheless, the number of binding sites appears to be lower than those of other furocoumarins (range: 0.7-2). In most studies, the number of binding sites is correlated with the liposolubility of the molecule; in our case, the difference of solubility between 3CPS and other psoralens appears too low to explain such a difference (3.8×10^{-5} M^{-1} for 3CPS, 2.3×10^{-5} M^{-1} for 8MOP and 2.7×10^{-5} M^{-1} for 5MOP). This low value of N could be explained by interference with free fatty acids: the same assay achieved with defatted HSA shows an increase of the number of binding sites (N = 0.47), which is twice the value obtained for the non defatted albumin, whereas K remains almost constant (K = 8.6×10^4 M^{-1}). FFA could alter the structure of the binding sites of 3CPS by a non competitive way, perhaps by inducing structural change of HSA (7); the non ionic character of 3CPS and the fact that K remains constant indicates that binding of FFA takes place on another site. Interactions between FFA and 3CPS have been already reported for other compounds (7, 17).

Using defatted HSA solution spiked with FFA the K value is constant (8.65×10^4 M^{-1}) but N decreases to 0.27, confirming the interference of FFA on 3CPS binding.

Table II. *Binding percentages of 3CPS to serum proteins compared with crude and defatted HSA (albumin concentration = 40 g/l).*

3CPS concentrations			Crude	Deffated
μM	μg/ml	Serum	HSA	HSA
0.38	0.1	89.9	74.6	90.8
1.94	0.5	91.7	75.5	91.4
3.87	1.0	91.4	74.1	90.5

Binding of 3CPS on serum proteins in physiological conditions and comparison with binding to crude and defatted HSA. — The results of binding of 3CPS to human serum proteins and to crude and purified HSA (albumin concentration = 40 g/l) are compared in table II. An important binding to defatted HSA (90%) is noticed, a lower one to crude HSA (75%), and a binding of 90% to total serum proteins, which is the sum of binding to HSA and to globulins. K and N were found to be respectively 8×10^4 M^{-1} and 0.064. It should be pointed out that the binding to crude HSA for 3CPS concentrations ranging 0.1 up to 2 μg/ml is lower than for other psoralens (TMP: 90%, Psoralen: 85%) (1, 15). The same affinity constant of 3CPS for concentration of 40 and 2 g/l HSA suggests that a saturable process occurred. A drastic decay of N indicates a very important alteration by FFA due to the relative higher concentration of FFA with 40 g/l HSA.

In therapeutics, a decrease of albumin in binding by FFA could explain some clinical failures observed in hyperlipaemic patients: the free fraction is most probably increased, thus enhancing the metabolism of the drug and decreasing the amounts of active 3CPS in the skin.

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Resumen

Se estudian las características de la unión del 3CPS a la albúmina humana y a las proteínas de suero humano. El estudio electroforético muestra que la albúmina es la fracción proteica a la que se une de forma predominante el 3CPS, y a las globulinas en menor medida. La unión a la albúmina, estudiada por diálisis de equilibrio, es del 75% y se caracteriza por un bajo número de sitios de unión saturables (N = 0,27), con una moderada constante de afinidad ($K = 8 \times 10^4$ M^{-1}). Los ácidos grasos libres disminuyen la unión del 3CPS a la albúmina, por un mecanismo no competitivo.

Palabras clave: 3-Carbetoxipsoraleno, Unión al suero, Unión a la albúmina.

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