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In vitro study of the protein binding of fusidic acid: a contribution to the comprehension of its pharmacokinetic behaviour

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Abstract

Fusidic acid (FA: Fucidine® Leo Laboratories) is a type I drug: acid, ionized at plasma pH, with a high intrinsic affinity for albumin. In the case of this kind of product, the possibility exists of the saturation of protein binding and of drug interactions. The relative affinity of fusidate (F) for different solutions of albumin was compared in vitro, i.e., purified industrial albumin, fresh frozen plasma, and plasma from surgical intensive care unit (ICU) patients. The theoretical consequences of the albumin status of a patient on FA kinetics were envisaged in the form of a model. The three stock solutions were diluted such that solution S1 yielded concentrations of 38, 29 and 19 g/l of albumin, and S2 provided concentrations of 44, 32 and 20 g/l of albumin. The concentration of solution S3 was initially 24 g/l (severe hypoalbuminemia). The seven solutions were spiked with known quantities of FA (10, 50, 100 and 150 mg/l) in which total F (tF) and free F (fuF) were assayed by HPLC. The per cent binding of F to albumin was determined, as was the number of binding sites (n) and its saturable or non-saturable character as a function of the medium (Scatchard plots). The conclusions drawn were: (1) the number of F binding sites on albumin is between two and three and the per cent binding of F is in the range of 91.5–98.7%; (2) Fucidine® belongs to the group of drugs sensitive to the albumin status of the media in which it is added; (3) the often severe hypoalbuminemia of ICU patients may lead to a considerable increase in the active fraction fuF; the most seriously affected patients should be more exposed to variations in the tF/fuF ratio; (4) the clinical status of the patients (kidney failure, malnutrition hypoalbuminemia, etc.) should be taken into account when determining the dosage schedules of parenterally administered FA. In summary, after repeated administration, the significant increase in fuF should induce the following: an increase in V_d, an increase then a decrease in Cl, a decrease then an increase in the AUC. The hypothesis according to which Fucidine® would be more effective in the hypoalbuminemic patient remains to be confirmed in selected patients with coupled measurements of tF anf fuF.

Keywords: Fusidic acid; In vitro study; Protein binding; Pharmacokinetics; HPLC

1. Introduction

Fusidic acid (FA) is an antistaphylococcal antibiotic whose chemical structure is related to that of steroids (Godtfredsen et al., 1962). The

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increasing frequency of severe hospital-acquired infections caused by multi-resistant staphylococci explains the re-evaluation of FA (Kraemer et al., 1982; Coombs and Menday, 1985; Faber and Rosdhal, 1990). Even though Fucidine® has been marketed for several years and is attracting renewed interest particularly in the injectable form, clinical and above all pharmacokinetic trials concerning this compound remain sporadic. Recently, Taburet et al. (1990) partially filled in these gaps by performing a study in healthy subjects of the pharmacokinetics of FA, administered intravenously and as a new enterocoated oral form. On the other hand, Bourget et al. (1993) have recently studied the pharmacokinetics of FA after a single dose of a new paediatric suspension. It is evident that the metabolic status, in particular concerning proteins, of patients in intensive care units is often characterized by a more or less severe hypoalbuminemia (Ghoneim and Kortilla, 1977; Wood, 1986). This is why transposing administration schedules established in healthy patients to the category of hospitalized patients is sometimes problematic, if not haphazard. For example, it is known that hypoalbuminemia increases patient sensitivity towards certain psychotropic drugs, such as diazepam (Bourget et al., 1992).

Fusidate (F) is ionized at plasma pH, with a normal high intrinsic affinity for albumin. It thus circulates 97–98% bound to albumin when the protein status of subjects is normal (Godtfredsen and Vangedal, 1966; Guttler et al., 1971). The aim of this study was to compare the relative affinity of F for various categories of albumin solutions in vitro, including plasma samples collected from hypoalbuminemic patients in surgical ICU. A metabolic model is proposed in order to evaluate the theoretical consequences of the albumin status of a patient on the kinetics of F and to better control its prescription or, more generally, that of similar drugs.

2. Experimental

2.1. Preparation and definition of working solutions

The concentrations of total and free F were measured in seven dilutions prepared from the following three stock solutions: (1) a solution of industrial plasma albumin (S1) (Biotransfusion, 59000 Lille, France); (2) fresh frozen plasma (FFP in 250 ml pouches) (Centre National de Transfusion Sanguine); and (3) a pool of 15 plasma samples (50 ml per patient and mixed in equal volumes) from patients in ICU (surgical intensive care unit of our center). None of the patients whose plasma was used to prepare solution S3 presented a picture of acute or chronic liver failure, known to modify the protein binding of a drug, sometimes significantly (quantitatively and/or qualitatively). Solutions S1–S3 were frozen at −80°C until assay.

Solutions S1 and S2 were diluted in isotonic saline (0.9% NaCl) such that six dilutions were obtained, containing of the order of 40 (S1a and S2a), 30 (S1b and S2b) and 20 (S1c et S2c) g/l of

Table 1
Comparative changes (mean values) in albumin binding of fusidate as a function of its concentration in the seven working solutions studied (from 10 to 150 mg/l)

Working solutions (S) (g/l of albumin)	Albumin binding of fusidate (%) at concentration of fusidate (mg/l):			
	10	50	100	150
S1a (38)	97.6	97.8	97.6	97.5
S1b (29)	97.4	97.0	96.5	95.9
S1c (19)	95.8	96.0	94.6	92.8
S2a (44)	97.1	98.7	98.3	97.4
S2b (32)	96.4	96.4	96.5	96.4
S2c (20)	95.2	95.9	93.5	92.6
S3 (24)	94.1	93.9	92.9	91.5

albumin. Solutions S1a and S2a correspond to a normal albumin status, while solutions S1b and S2b represent a moderate, and S1c and S2c to a severe hypoalbuminemia (often encountered in ICU patients). Solution S3 was not diluted, since its initial albumin concentration was 24 g/l and thus initially represented an actual situation of severe hypoalbuminemia. The exact albumin concentrations of the seven working solutions were determined by protein electrophoresis. The seven working solutions were spiked with sodium fusidate (solution containing a precise concentration of fusidic acid) at four concentrations per solutions, i.e., 10, 50, 100 and 150 mg/l. This range of concentrations is equivalent to those usually encountered in the blood of subjects treated parenterally with Fucidine® for injection (Reeves, 1987; Taburet et al., 1990). Thus, a total of 28 final working solutions were examined. All measurements were carried out after temperature equilibration at 37°C (2 h incubation in a water bath). The concentrations of total (tF) and free (fuF) were determined five times in each of the 28 final working solutions.

2.2. Assay method: determination of total and free fusidate

Concentrations of total F were determined by high-performance liquid chromatography combined with UV spectrophotometric detection at 235 nm. The technique used complied with the analytical recommendations of Sørensen (1988). Fusidic acid and 24,25-dihydrofusidic acid, used as an internal standard, were kindly provided by Léo Laboratories (Montigny-le-Bretonneux, France). The method used a test specimen of 100 μl of serum and its limit of detection was 1 mg/l. The accuracy of the assay method, studied at the target values of 5, 25 and 100 mg/l (serum loaded in vitro with fusidic acid), gave coefficients of variation of 8.3, 3.5 and 2.3, respectively. This system provided good separation of the compounds corresponding to the following retention times, i.e., 7.7, and 9.9 min for fusidate and internal standard, respectively. It is important to point out that, under the analytical conditions described above, samples do not need any pretreatment before the extraction procedure. It is important to stress that HPLC is the technique of choice for fusidic acid and because of its accuracy and high level of specificity, it is preferable, as has been emphasized by Reeves (1987), to the microbiological determination of concentrations.

The extent of fusidate binding to proteins was determined with an ultrafiltration system. The Ultrafree® CL device (ref. UFC4LG25, Millipore, Saint-Quentin-en-Yvelines, France) was used (Ohshima et al., 1988). In brief, 1 ml of the sample (i.e., plasma) was placed in the filter cup (upper part of the device) which is separated from the filtrate cup by an ultrafiltration membrane. Ultrafiltration was achieved by nitrogen under pressure (4 kg/cm^2). The device was kept under these conditions for 2 h (at 37°C). In ultrafiltrate (filtrate cup) the concentrations of unbound fusidate were determined by the same method as mentioned above.

2.3. Data processing

The per cent binding of F to albumin was calculated with the following formula (Singlas, 1987):

$$\text{binding}(\%) = (\text{tF} - \text{fuF}/\text{tF}) \times 100 \quad (1)$$

Since binding to proteins is a reversible phenomenon, governed by the law of mass action, we can write:

$$[\text{M}] + [\text{P}] \underset{k_2}{\overset{k_1}{\rightleftarrows}} [\text{MP}] \quad (2)$$

in which [M] is the concentration of the free drug, [P] the concentration of free proteins and [MP] the concentration of the drug bound to proteins. At equilibrium, the association or affinity constant K_a is defined as:

$$K_a = k_1/k_2 = [\text{MP}]/([\text{M}] + [\text{P}]) \quad (3)$$

If r is the ratio between the molar concentration of the bound drug [MP] and the molar concentration of proteins ([MP] + [P]), we can write:

$$r = ((K_a \times [\text{M}])/(K_a \times [\text{M}]) + 1) \quad (4)$$

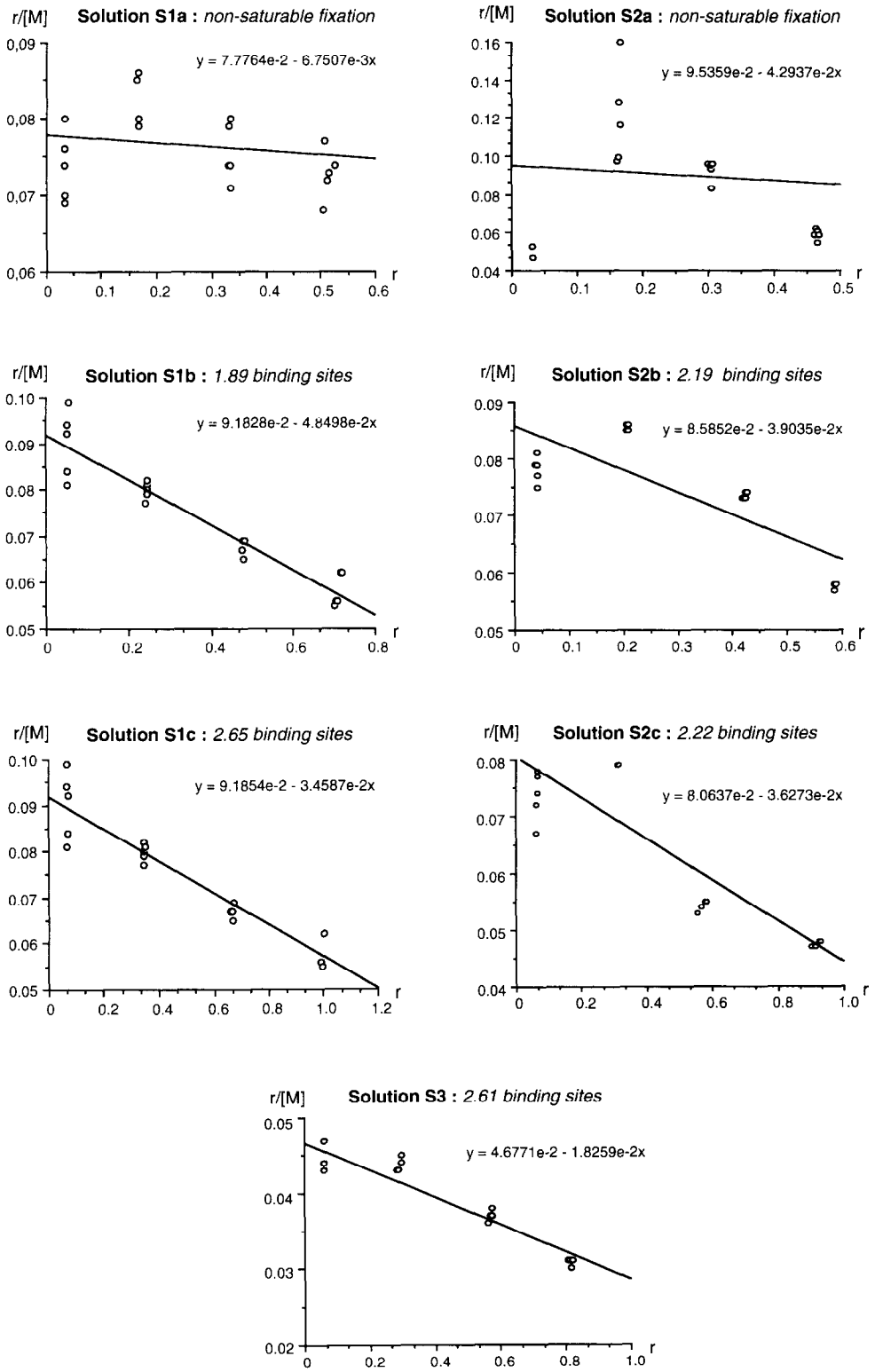

Fig. 1. Scatchard plots of albumin binding of fusidate in each of the seven solutions studied.

If for a given drug there exists a number n of identical and independent binding sites on a protein, we can write:

$$r_1 + r_2 + \cdots + r_n = r_{\text{total}}$$
$$= n \times ((K_a \times [M])/(K_a \times [M]) + 1) \quad (5)$$

By conducting measurements for several drug concentrations, we can thus calculate [M] and r. When the results are plotted graphically, K_a and n can be determined. Among the methods available, the Scatchard plot is often used after changing variables (Scatchard, 1949). After transformation, it uses Eq. 5:

$$r/[M] = n \times K_a - r \times K_a \quad (6)$$

Plotting $r/[M]$ vs r, we obtain a line after linear regression whose y intercept is $(n \times K_a)$, whose slope is K_a, and whose x-intercept determines n (Scatchard, 1949; Singlas, 1987).

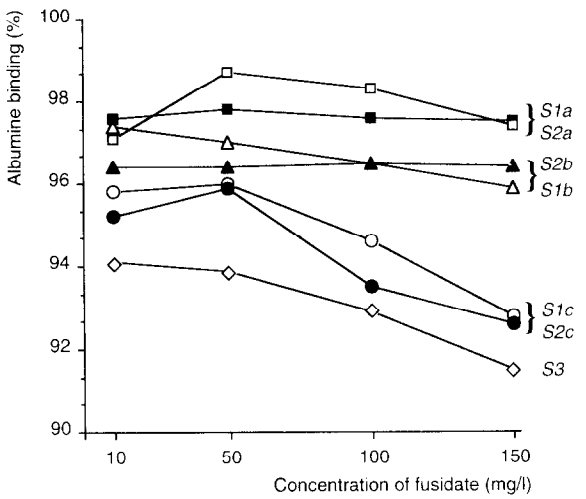

Fig. 2. Comparative changes (Cartesian coordinates) in albumin binding of fusidate as a function of its concentration in the seven solutions studied.

3. Results and discussion

Table 1 lists the mean per cent binding of F to albumin as a function of antibiotic concentration for the seven solutions. This per cent binding varied from 91.5% for $S3_{150\ mg/l}$ to 98.7% for $S2a_{50\ mg/l}$. Per cent albumin binding in solutions S1 and S2 decreased as the protein concentration decreased, independently of the antibiotic concentration (from 10 to 150 mg/l). In other terms, the free fraction fuF increased with the severity of the hypoalbuminemia, whether in purified industrial solutions or normal human solutions (FFP). For S3 (hospitalized patients), however, the fuF fractions were always higher than those of solutions S1c and S2c, regardless of the antibiotic concentration.

Fig. 1 shows Scatchard plots of the seven solutions of $[r/[M] = f(r)]$. Fig. 2 enables the relationships [albumin binding (%) $= f$(concentration of F)] to be compared among the seven solutions. Three very similar pairs of profiles are shown by this representation: profiles S1a and S2a (normal albumin concentrations), S1b and S2b (moderate hypoalbuminemia) and finally S1c and S2c (severe hypoalbuminemia). The situation is special for S3: this pathologic solution, whose albumin concentration is about 20% higher than that of solutions S1c and S2c, has a considerably reduced protein binding capacity for F.

Concerning solutions S1a and S2a (normoalbuminemia), fusidate binding to albumin was non-saturable with the concentrations tested, while it could be saturated at low or intermediate protein concentrations (solutions S1b, S1c, S2b, S2c and S3). Thus, when possible the number of F binding sites, 'n' was determined, and was between 2 and 3 (from 1.89 for S1b to 2.65 for S1c), consistent with the few published data on this subject (Guttler et al., 1971). For solution S3, the number of binding sites was normal. Information provided by measurement of total and free fusidate may be analysed on the basis of the following features.

The binding of F to albumin exceeds 97% when patient protein status is normal. This is also true in vitro for the solution of purified albumin (solution S1a) and the FFP (solution S2a). The characteristics of fusidate make it a so-called type I drug. The combination of a reduced number of sites (< 4) and high affinity suggests a possible saturation of protein binding and the appearance of drug interactions (Labaune, 1988). These basic

data are to be compared to the possible appearance of protein 'unbinding', or release, resulting from a metabolic disorder (malnutrition, stress, etc.) and/or a polymedication. The latter could theoretically affect the distribution, metabolism, elimination rate and even the activity of the drug (Singlas, 1987; Rowland and Tozer, 1989). The potential clinical consequences would depend in particular on the therapeutic spectrum of the product, its intrinsic safety and its therapeutic index. For a given binding deficit, the free active fraction (fuF) is higher as initial protein binding was greater. In other terms, the release of several per cent considerably increases the therapeutic potential of fusidate. As an example, F was bound to albumin in solution $S1c_{150\ mg/l}$ at 92.8%. This binding increased to 97.5% in solution $S1a_{150\ mg/l}$. Between these two values, the free active fraction of F increased by 188%. Similarly, between solution $S2a_{150\ mg/l}$ and solution $S3_{150\ mg/l}$ (hospitalized patients), the pharmacologically active fraction increased by 226%.

It is probable that the increase in fuF during severe albumin depletion in patients will affect all the metabolic steps related to the drug.

Distribution step: In a normoprotein situation (healthy volunteers), it has been shown that the apparent volume of distribution (V_d) of F is relatively low, of the order of 0.30 ± 0.04 l/kg after administration (infusion) of an initial dose of 500 mg and is then 0.21 ± 0.02 l/kg after the ninth dose (Taburet et al., 1990). The consequences of protein release on the distribution of fusidate are directly dependent on its relative affinity for tissue proteins in comparison to blood proteins. Thus, high tissue affinity can be considered as an active diffusion mechanism. Since only the nonionized liposoluble fraction of the drug can cross the different tissue membranes, a number of parameters participate (pH of media, vascularization, relative composition of tissues, lipid/water partition coefficient, etc.). For a drug whose V_d is small and the result of vascular retention due to high protein affinity, an increase of fuF, even if only moderate, will have considerable effects on tissue distribution. In hypoalbuminemic patients, it is expected that there will be a more or less clearcut increase in the V_d of fusidate in vivo.

Metabolism and elimination: Fusidate is practically totally excreted in the bile in metabolized form and only 2% of the dose is excreted unchanged (Godtfredsen et al., 1979; Rowland and Tozer, 1989). The total clearance (Cl) of fusidate is thus essentially hepatic clearance. In addition, its coefficient of hepatic extraction is low ($E_H < 0.30$) meaning that it easily passes through the liver.

Three parameters define the hepatic uptake of F (and of a drug in general): (1) the rate of hepatic blood flow which determines the supply of the drug to the purification organ; (2) the intrinsic enzymatic activity expressed by hepatic clearance; and (3) protein binding itself since only fuF can diffuse into the liver cells. In the case of drugs with a low coefficient of extraction, the affinity for hepatic tissue is always lower than that for blood proteins. This is important since the coefficient of extraction will remain low, whether or not a large or small quantity of drug reaches the liver (Schary and Rowland, 1983). If for a drug, however, fuF increases, then liver cell uptake and thus Cl increase. In other terms, protein binding limits elimination only of those drugs whose coefficient of extraction is low, i.e., those having a reduced affinity for elimination organs (Godtfredsen and Vangedal, 1966; Faber and Rosdhal, 1990; Taburet et al., 1990). This is true, however, only if the liver can cope with the metabolic overload. In the case of fusidate, it has been shown that its hepatic metabolism can be saturated, thereby introducing the concept of nonlinear kinetics and explaining the fairly frequent appearance of jaundice, most often without gravity. In practice, it is consistent to think that although initially (first administrations) antibiotic clearance increases (increased hepatic uptake of fuF following protein release), once hepatic clearance capacity is saturated and then exceeded the same clearance decreases and finally the area under the curve of concentrations (AUC) should increase.

Functional qualities of transport proteins: Protein release measured for solution S3 (hospitalized patients) was, for the same concentration of F and proteins, somewhat more pronounced than the release determined in normal or comparable

plasmas. This phenomenon could be related to the presence of moderate liver failure in 10 of the 15 patients selected. It has been shown that release is correlated to the degree of renal impairment, determined by examining creatinine clearance or blood urea (Gugler et al., 1975). Kidney failure, even moderate, often causes hypoalbuminemia whose results in terms of release are considerate for type I drugs. Among other things, it is often associated with a more or less pronounced loss of albumin functionality. Varying degrees of conformational changes have been identified using special electrophoretic analysis methods (diminished B band in kidney failure patients) (Boobis, 1977). The reduced functionality of this pathological albumin would explain why, even though the albumin concentration of solution S3 was 20% higher than that of solutions S1c and S2c, release was more pronounced.

Possible results on the efficacy of fusidate: A hypothesis can be proposed concerning the result of severe hypoalbuminemia on the kinetics and therapeutic efficacy of fusidate. If fuF and thus the V_d of F increase in the course of treatment, while hepatic clearance capacities are rapidly saturated, it may be considered that the liver is in a situation of metabolic strangulation. In this case and for a patient with the same body weight receiving the same dose of fusidic acid, it is reasonable to consider that the tissue concentrations of antibiotic will be higher if hypoalbuminemia is present. Thus, paradoxically, fusidate would be virtually more effective in this patient category.

In summary, theoretical data processing enables the elimination of various factors that may significantly modify the conclusions of an observation but does not permit the unambiguous deduction that it will occur in vivo as a result of the behavior of a product, in particular in a situation of stress or of septic aggression. Regardless of the actual situation, this work has shown that: (1) fusidic acid belongs to the group of drugs sensitive to the albumin status in which it is placed; (2) the often severe hypoalbuminemia of ICU patients can cause a considerable increase in the active fraction, fuF; (3) the most seriously affected patients should be more exposed to variations in the tF/fuF ratio, a notion that is especially important as a result of its consequences; and (4) patient status (kidney failure, malnutrition hypoalbuminemia, etc.) should be considered when defining FA dosage schedules for parenteral administration. In summary, the significant increase in fuF should lead to the following after repeated administration: an increase in V_d, an increase followed by a decrease in Cl, and a decrease then an increase in the AUC.

The hypothesis according to which fusidate would be more effective in the hypoalbuminemia patient requires confirmation in selected patients, with coupled measurements of tF and fuF. This would enable clinicians to envisage more optimized dosage schedules, in light of real situations encountered in IC units. Finally, beyond the specific question of FA, this relatively inexpensive approach in vitro could constitute a useful tool for initial investigations with the aim of better control of certain therapies.

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