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Note

Study of the protein binding of fusidic acid in cholestatic individuals and comparison with in vitro findings

P. Bourget[a,*], V. Quinquis-Desmaris[a], A. Hulin[a], J.D. Peter[b], F. Jehl[c]

[a]*Laboratory of Pharmacology and Toxicology, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75743 Paris, France*
[b]*Department of Anesthesiology, Hôpitaux Universitaires de Strasbourg, Strasbourg, France*
[c]*Laboratory of Pharmacokinetic, Institut de Bactériologie, Strasbourg, Strasbourg, France*

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Abstract

We have recently studied the relative affinity of fusidate (F) for various categories of albumin solutions in vitro, including plasma samples collected from hypoalbuminemic patients in surgical ICU. A metabolic model has been proposed in order to evaluate the theoretical consequences of the albumin status of a patient on F kinetics. On the other hand, it has been suggested by Peter et al. (1993) that pharmacokinetic changes observed for FA in patients with cholestasis are related to an alteration of the metabolism of the free fraction of fusidate (fuF), i.e. a marked increase, linked with the hypoalbuminemic status of the subjects. In order to verify in part our hypothesis and the one suggested by Peter et al. (1993), we studied the behaviour of the unbound fraction of F administered in the cholestatic individuals previously studied. Data obtained in vitro were compared to those determined after pharmacokinetic analysis in patients with cholestasis: (1) there was a significant increase in C_{max} and C_{min} of total and unbound F between the first and 10th infusion; (2) AUCs of fuF did not differ between groups of subjects; it could be considered that, at equal dose, behavior of fuF is not dependent upon the type of disease; (3) hypoalbuminemia cannot alone explain the marked increase in fuF seen in patients. We feel that results obtained in vivo are the product of hypoalbuminemia and of a picture of liver failure. Cholestasis in itself does not appear to play a preponderant role. In total, comparison of data obtained in this study shows the limitations of certain in vitro models.

Keywords: Fusidic acid; Protein binding; In vitro analysis; Cholestatic subjects; Pharmacokinetics; HPLC

The increasing frequency of severe hospital-acquired infections caused by multi-resistant staphylococci explains the re-evaluation of fusidic acid (FA; Fucidine® Leo Laboratories) (Kraemer et al., 1982; Coombs and Menday, 1985; Faber and Rosdhal, 1990; Taburet et al., 1990; Bourget et al., 1993a). Fusidate (F) is ionized at plasma pH, with a normal high intrinsic affinity for albumin. It thus circulates 97–98% bound to albumin when

* Corresponding author. Service de Pharmacie Clinique, Laboratoire de Toxicologie. G. H. Necker-Enfants Malades, 149 rue de Sèvres, 75743 Paris Cédex 15, France.

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the protein status of subjects is normal (Godtfredsen et al., 1979). 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).

Peter et al. (1993) have recently studied the pharmacokinetics of intravenous FA in patients with cholestasis. The pharmacokinetics of FA and its main metabolite, 3-keto FA, were examinated after single and repeated intravenous administration in postoperative cholestatic patients and in postoperative noncholestatic patients. It has been suggested that pharmacokinetic changes observed in patients with severe cholestasis are related to an alteration of the metabolism of the free fraction of fusidate (fuF) linked with the hypoalbuminemic status of the subjects. On the other hand, we have recently studied the relative affinity of F for various categories of albumin solutions in vitro, including plasma samples collected from hypoalbuminemic patients in surgical ICU (Rieutord et al., 1995). A metabolic model has been proposed in order to evaluate the theoretical consequences of the albumin status of a patient on F kinetics. To verify in part our hypothesis and one suggested by Peter et al. (1993), we studied the behaviour of fuF in the cholestatic patients previously studied.

1. In vitro study.

The details of the work are presented in the paper previously published (Rieutord et al., 1995). In brief: the concentrations of total (tF) and free (fuF) F were measured in 7 dilutions prepared from 3 stock solutions: (1) a solution of industrial plasma albumin (S1); (2) fresh frozen plasma (FFP) (S2); and (3) a pool of 15 plasma samples from patients in ICU (S3). Solutions S1 and S2 were diluted (0.9% NaCl) such that 6 dilutions were obtained, containing of the order of 40 (S1a and S2a), 30 (S1b and S2b) and 20 (S1c et S2c) g/l of albumin. Solutions S1a and S2a correspond to a normal albumin status, while solutions S1b et S2b correspond to 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, i.e. a situation of severe hypoalbuminaemia. The 7 working solutions were spiked with sodium fusidate at 4 concentrations per solutions, i.e. 10, 50, 100 and 150 mg/l. Thus, 28 final working solutions were examined.

2. In vivo study.

Once again, the details of the work by Peter et al. (1993) are presented in their paper previously published. In brief: (1) 21 postoperative patients suffering from pneumonia or septicemia caused by FA-susceptible staphylococcal strains participed in the study; they were divided in 3 groups similar with respect to age, weight and prognostic indices. Patients with acute liver disease and severe hepatic impairement were excluded from the study; (2) Group I included 6 nonicteric subjects with conjugated bilirubin (μmol/l) and serum alkaline phosphatase (IU/l) laboratory values within the normal ranges (i.e., 4.8 $\pm$ 2.6 and 83.8 $\pm$ 35.8, respectively). Group

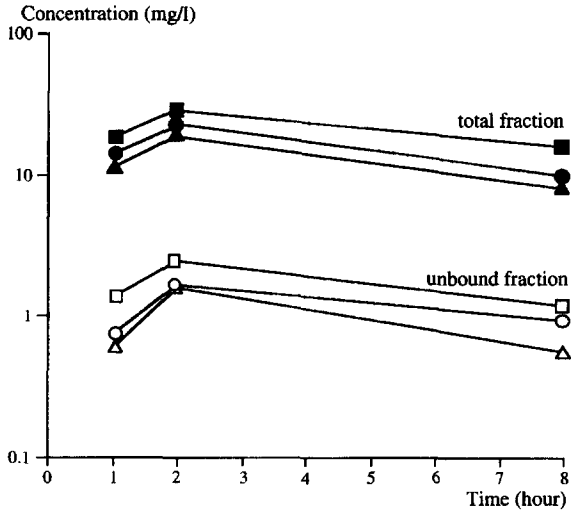

Fig. 1. Comparative changes (semilogarithmic coordinates) in the plasma concentrations of total (closed symbols) and unbound (open symbols) fractions of fusidate after the first 500 mg infusion in 3 groups of hospitalized patients, i.e. Group I (circles, n = 6); Group II (triangles, n = 9); Group III (squares, n = 6).

Table 1
Comparison of mean (± S.E.M.) pharmacokinetic parameters for total and free fractions of fusidate obtained in the three groups of patients after the first 500 mg infusion (D1) and at plateau (D10)

Parameters	Group I (n = 6)		Group II (n = 9)		Group III (n = 6)	
	D1	D10	D1	D10	D1	D10
Total fraction						
C_{max} (mg/l)	24.50 ± 13.25[a]	58.60 ± 19.78[a]	22.2 ± 4.5	44.9 ± 12.2	32.6 ± 6.6[b]	92.2 ± 22.6[b,c]
C_{min} (mg/l)	8.90 ± 5.44	38.72 ± 16.30[a]	7.9 ± 2.2	21.1 ± 9.6	16.4 ± 4.6[b,c]	65.4 ± 22.6[b,c]
AUC1 → 8 (mg·h/l)	134.98 ± 65.95[a]	—	112.7 ± 20.9	—	179.9 ± 41.5[b]	—
Unbound Fraction						
C_{max} (mg/l)	1.7 ± 0.9[d]	5.7 ± 3.4	1.6 ± 0.8[d]	3.6 ± 2.1	2.5 ± 0.6[d]	8.4 ± 2.9
C_{min} (mg/l)	0.9 ± 0.7[d]	3.6 ± 2.2	0.6 ± 0.3[d]	2.0 ± 1.1	1.2 ± 0.3[d]	5.5 ± 2.2
AUC1 → 8 (mg·h/l)	9.54 ± 4.90	—	8.4 ± 3.8	—	14.0 ± 3.7	—
Albumin binding of fusidate (%)	92.8 ± 2.2	—	92.4 ± 3.4	—	92.0 ± 2.6	—

C_{max}, maximum drug concentration; C_{min} minimum drug concentration; AUC1 → 8 area under the concentration-time curve 1 → 8, i.e. approximated AUC based on three measurements after D1.[a]$P<0.05$ vs. Group II.[b]$P<0.05$ vs. Group II.[c]$P<0.05$ vs. Group I.[d]$P<0.05$ vs. 10th infusion.

II included 9 patients with bilirubin, alkaline phosphatase, and 5′-nucleotidase activities above the upper limits of normal (i.e. 47 ± 44, 280 ± 182.5 and 7.5 ± 3.8, respectively). Group III included 6 patients with isolated conjugated hyperbilirubinemia (i.e. 98.1 ± 71.9); (3) the patients presented a similar hypoalbuminemic status, i.e. 29.7 ± 5.2, 26.3 ± 3.9 and 29.2 ± 3 g/l respectively for Group I, II and III. These values are closely to those of the solutions S1b and S3 prepared for in vitro analysis, i.e. 29 and 24 g/l respectively; (4) FA was given in combination with either an aminoglycoside or a glycopeptide, i.e. 500 mg intravenously over 2 h, every 8 h for at least 4 days.

To perform the pharmacokinetic analysis of both total and unbound fractions of F (i.e. tF and fuF respectively) in the 3 groups of patients, 1 series of arterial blood samples was collected after the start of infusion D1, i.e. at time 1.0, 2.0 (C_{max}D1) and 8.0 h (C_{min}D1). Two arterial blood samples were also withdrawned at plateau following the 10th dose (D10), i.e. at time 2.0 (C_{max}D10) and 8.0 h (C_{min}D10). The area under the curve ($AUC_{1\rightarrow 8}$, i.e. *approximated* AUC) was calculated by the trapezoidal rule from time '1' to time 't = 8 h' (Bourget and Delouis, 1993b). Thus, to determine the mean per cent binding of F to albumin, AUCs were calculated for tF and the corresponding fuF. F binding kinetics were also studied. The per cent binding of F to albumin was calculated with the following formula: Binding (%) = (tF − fuF/tF) · 100.

Concentrations of both tF and fuF were determined by high performance liquid chromatography combined with UV spectrophotometric detection at 235 nm (Sørensen, 1988). The extent of fusidate binding to proteins was determined with an ultrafiltration system. The Ultrafree® CL device (Millipore, Saint-Quentin-en-Yvelines, France) was used (1 ml of plasma lead to 200 μl of ultrafiltrate) (Ohshima et al., 1988).

Kinetic data determined for tF and fuF following the first 500 mg dose of F were compared by distribution free Wilcoxon T test; C_{max} and C_{min} values measured at plateau were also compared by using this same test. A distribution-free Mann-Whitney test was used to compare tF vs fuF values obtained in the 3 groups. Linear regression, by using the method of least squares, was used to assess relationships between pharmacokinetic parameters and biological and morphometric data, with statistical significance determined using the F test. A P value of < 0.05 was considered to be statistically significant.

Fig. 1 shows, decreases in concentrations of tF and fuF in relation to time, for each of the 3 groups of patients; Table 1 compares the corresponding pharmacokinetic parameters. There was a significant increase in C_{max} and C_{min} tF and fuF between the first and the 10th infusion. This must be viewed in the context of the accumulation of F during treatment described by various authors (Reeves, 1987; Taburet et al., 1990). AUCs of fuF did not differ between groups of subjects and it could be considered that, at equal dose, behavior of the unbound fraction is not dependent upon the type of disease. Further, overall review of the 21 subjects revealed the existence of a link between serum albumin or alkaline phosphatase levels and $C_{min}D1$, as well as between direct bilirubin and percentage binding of F to albumin (Fig. 2).

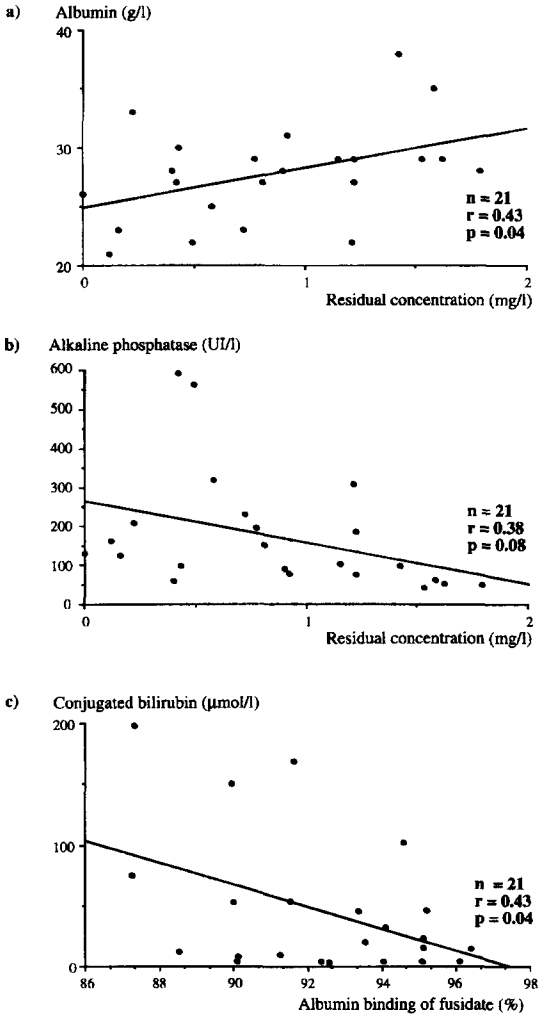

Fig. 2. Relationships between the minimum fusidate concentration (CminD1) measured after the first 500 mg infusion and either albuminemic status of the 21 patients (a) or alkaline phosphatase (b) and between albumin binding of fusidate and the conjugated bilirubin (c).

Fig. 3 illustrates relations between albumin levels in the media examined (i.e. in vivo and in vitro) and corresponding binding rates of F (fuF) to albumin. Data determined in vitro are shown alongside results obtained in the 3 groups of subjects when they were of subsequent interest. Thus, binding levels of a substance such as F depend upon albumin levels, the concentration of F (from 10 to 150 mg/l) and the nature of media. The trend was as follows: (1) protein binding levels were all the lower when albumin was low and the concentration of F high; (2) solution S1a provided the expected reference value when albumin status was normal (of the order of 38 to 40 g/l), with saturation binding regardless of the concentration of F; and (3) S3 was a special case in which binding levels were lower than would have been expected, in view of a serum albumin of 24 g/l; the hypothesis of a modification of the functionality of S3 albumin was suggested (Boobis, 1977). Two remarks must be made: (1) solution S1b had an albumin status (29 g/l) of the same order of that of the patients, and (2) solution S3 was closest to patients in terms of its origin. However, in both situations, control and cholestatic subjects showed binding levels notably lower than those measured for S1b and even for S3, in particular taking into account the lowest concentrations of F (i.e. 10 and 50 mg/l) which are also encountered in vivo. In vitro study of protein binding of drugs using solutions with a composition different from that of physiologic media (e.g. S1b) cannot fully reproduce pathophysiologic reality. Thus solutions S1c and S2c, despite their titration of 19 and 20 g of albumin per liter, respectively, showed very low binding levels only at high concentrations of F (i.e. 100 and 150 mg/l), never encountered in vivo. Hypoalbuminemia thus cannot alone explain the marked increase in fuF seen in patients. With regard to the hypotheses advanced by Peter et al. (1993) to

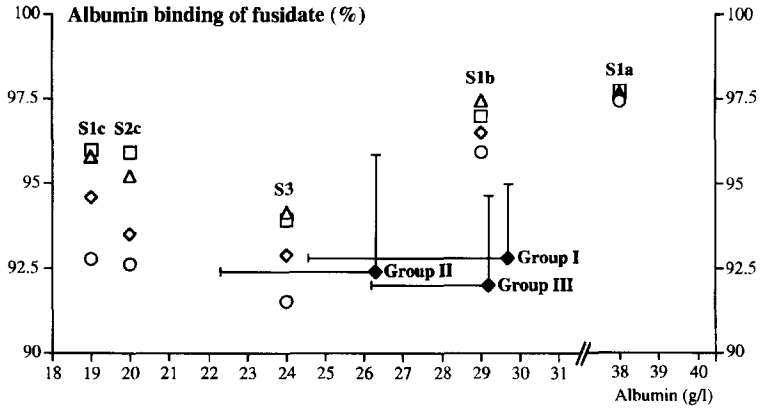

Fig. 3. Relationships (mean values) between the per cent binding of fusidate to albumin and the albuminemic status determined in vivo for the 3 groups of hospitalized patients (closed symbols ± S.E.M.) and in vitro study for solutions S1a (reference values), S1b, S1c, S2c and S3 spiked with sodium fusidate at 4 concentrations per solutions, i.e. 10 (triangles); 50 (squares); 100 (lozenges); 150 (circles) mg/l.

explain modifications seen in total F fraction, caution is necessary before concluding that only fuF is responsible since the behavior of this fraction is independent of pathophysiologic status, which was in fact fairly heterogeneous. Comparative analysis of liver function profiles of the 3 groups showed that total and direct bilirubin were the chief differential parameters, i.e. 10.9, ±,4.7, 72.1, ±,65.4 and 160.8, ±,112.2 μmol/l and 4.8, ±,2.6, 47, ±,44 and 98.1, ±,71.9 μmol/l in groups I, II and III, respectively. We feel that results obtained in vivo are the product of hypoalbuminemia and of a picture of liver failure, itself in moderate form, in several patients forming S3. Cholestasis in itself does not appear to play a preponderant role, at least in these moderate forms.

In total, comparison of data obtained in this study shows the limitations of certain in vitro models. For substances such as FA, the albumin status of subjects and their liver function seem to have a primordial influence on the behavior of fuF. In contrast, the existence of moderate cholestasis and circulating F levels appear to have no effect.

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