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High plasma protein binding as a parameter in the selection of betablockers for lactating women

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Many studies have been performed to determine, simultaneously, plasma and breastmilk concentrations of drugs after single or continuous doses in lactating women [1–5]. This has been the most frequently used approach in previous studies of the transfer of drugs from blood to breastmilk. It is generally considered that the main mechanism involved in drug transport from plasma to breastmilk is passive diffusion. Most weak acids and bases enter milk as the non-protein-bound, unionized forms to achieve concentrations that depend on the pH gradient between plasma and milk, the protein binding and the partition coefficient [1].

All these parameters are either known or may be estimated by *in vitro* experiments, thus it should be possible to forecast transfer of drugs into milk. The aim of this work therefore was to investigate whether *in vitro* studies can assist in the choice of drugs in lactating women, in order to avoid the cost and complexity of clinical studies.

Betablockers are frequently prescribed as hypotensive agents for young patients and are therefore often chosen for the treatment of hypertension during lactation. Consequently this class of compounds was selected for study.

Materials and methods

The milk binding of adrenergic beta-receptor antagonists was studied by equilibrium dialysis using the Dianorn® apparatus. The experimental conditions were 37°, pH = 6, constant stirring at 20 r.p.m. for 3.5 hr, at which time equilibrium was reached. Concentrations ranging from 0.25 to 250 μg/ml were used for each of the beta-blockers in 0.066 M phosphate buffer. They were prepared by isotopic dilution of a constant amount of labelled compound mixed with increasing amounts of unlabelled drug.

All labelled drugs [^{14}C]acebutolol (6.4 Ci/mole, Specia), [^{14}C]bornaprolol (44 Ci/mole, Pharmuka), [^{14}C]butofilolol (12 Ci/mole, CEA), [^{14}C]pindolol (22.5 Ci/mole, Sandoz), [^{3}H]propranolol (25,000 Ci/mole, Amersham), [^{3}H]sotalol (500 Ci/mole, CEA) have a chemical purity greater than 98%.

R_F values of the beta-adrenoceptor blocking drugs were determined by thin layer chromatography (TLC), using the following system of solvants: $CHCl_3/CH_3OH/NH_4OH$, 80:20:1.5, v:v.

Pooled human breastmilk was used (pH 6, 20 mM triglycerides). Milk fat was obtained by centrifugation for 30 min at 5000 *g* and resuspended in phosphate buffer at pH 6, then ultrasonicated.

At equilibrium the bound (B) and free (F) concentrations were measured and a curve B = f(F) was plotted. Since the binding was not saturable in the range of drug concentrations studied, no association constant *(K)* or binding sites concentration *(N)* could be derived from the data. The only binding parameter that could be estimated was a partition coefficient, *N*K, which relates the bound to free drug concentrations,

$$B = (NK).F \quad (1)$$

The data were fitted to equation (1) by a least squares regression program.

Results

The six drugs differ significantly in lipophilicity, as indicated by their R_f values upon TLC (Table 1). Their plasma binding is also related to the R_f values, i.e. the degree of lipophilicity.

Table 1 also summarizes the binding percentages obtained for these drugs in whole milk, whey and fat. The results indicate that the sum of binding percentages in whey and fat at an equivalent concentration of triglycerides, relative to milk, is superimposable to the percentage of overall binding in milk. The binding to milk is noticeably lower for the less lipophilic drugs, although they are highly concentrated in milk. Furthermore it is apparent that the bindings to both plasma and milk are positively correlated with the degree of lipophilicity.

Figure 1 illustrates the effect of pH on the binding of propranolol to milk. The milk binding, expressed in terms of the binding ratio B/F is correlated positively with the pH ($r_1 = 0.986$, $\alpha_1 < 0.01$) and negatively with the log value of the ratio of ionized to unionized drug, I/NI, assuming a pK_a of 9.5 ($r_2 = 0.983$, $\alpha_2 < 0.01$). As shown in Fig. 2, there are linear relationships between the R_f value and the log value of *NK* product of the drug, both in serum and in breast milk. The slopes of the respective curves, which exhibit this relationship in serum and in breastmilk, were not significantly different ($P > 0.20$).

Table 1. Physicochemical parameters of the drugs studied

Drug	R_f	Binding percentages in Plasma	Whole milk	Whey	Fat	Reported values of Drug $\frac{\text{Milk}}{\text{Plasma}}$ ratio
Sotalol	0.38	5	3	2	1	5.4 (6)
Acebutolol	0.45	27	11	2	9	7.1 (6)
Pindolol	0.49	50	23	4	15	1.6 (6)
Butofilolol	0.61	83	84	4	77	ND
Propranolol	0.67	93	88	4	84	0.4 (6)
Bornaprolol	0.72	98	99	4	95	ND

The coefficient of lipid solubility was determined by TLC using the following solvant system: $CHCl_3$/CH_3OH/NH_4OH, 80:20:1.5, v:v. The product NK was successively computed in plasma, whole milk, whey and on a suspension of fat in phosphate buffer from triglycerides concentration was similar to those of milk, i.e. 20 mM.

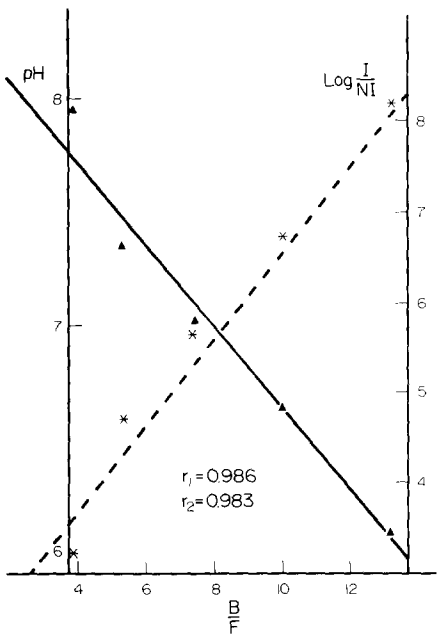

Fig. 1. Variation in the percentage of bound propranolol in breastmilk as a function of the corresponding pH and the ionization of the drug. The pH of the sample of pooled milk was 6. Variation in the pH of the milk from 6 to 8 was produced by addition of NaOH (0.1 M). The ratio I/NI was computed for propranolol assuming a pK_a of 9.5. Correlations between the milk binding of propranolol B/F and the ionization of the drug expressed by Log I/NI (▲) or the milk pH (*) are highly significant.

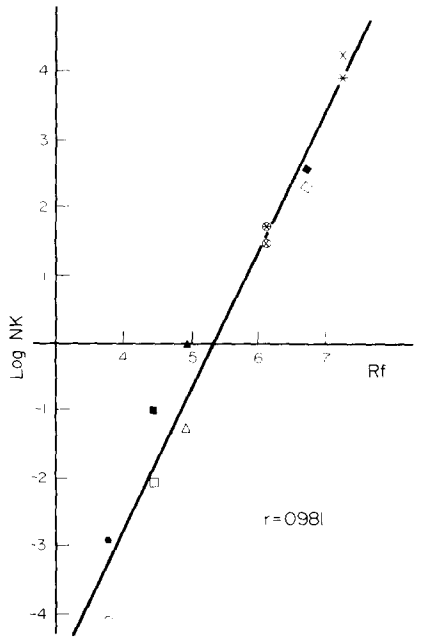

Fig. 2. Relationship between drug liposolubility and their binding products NK in serum (shaded symbols) and milk (open symbols). The liposolubility coefficient R_f was calculated for each drug after TLC using the following system of solvents: $CHCl_3$/CH_3OH/NH_4OH, 80:20:1.5, v:v, sotalol (●); acebutolol (■); pindolol (▲); butofilolol (⊙); propranolol (◆) and bornaprolol (*). The correlation between Log NK and R_f is highly significant ($r = 0.981$, $\alpha < 0.01$). The plasma and milk products NK are respectively determined at their physiological pH 7.4 and 6.

Discussion

The transfer of a drug from plasma to milk is theoretically related to the pK_a, partition ratio and plasma binding of the drug. Since only the non ionized fraction can diffuse across membranes, it is the concentration gradient of unionized drug between plasma and milk which should be considered. Weak acids are essentially completely ionized at plasma pH and thus diffuse rather slowly into milk. In contrast weak bases have a milk-to-plasma concentration gradient which is often greater than 1. The weakly basic beta-blockers have pK_a between 8 and 9.5. The variations of ionization between these drugs cannot therefore explain the great differences which are observed between their milk-to-plasma concentration ratios.

The partition coefficient of a drug determines both its penetration through the biological membranes and its concentration in milk fat. Our classification according to the lipid solubility of the drugs was similar to one described previously [7]. It is expected that the more lipophilic drugs would concentrate appreciably in milk by solubilization in the fat components. Such a mechanism can be justified, on the basis that the lipid concentration of milk is 6–9 times

greater than that of plasma [6]. Moreover Table 1 suggests that all the drugs dissolve in the fat components of milk. The milk whey, which contains the proteins of the milk, does not bind the drugs. The increase in milk localization, when the ionized fraction of the drug is smaller at higher pH, strengthens the hypothesis that the drug undergoes liposolubilization in triglyceride micelles of milk.

Notwithstanding these considerations of lipid solubility, the less lipophilic drugs are more extensively concentrated in milk (Table 1). This suggests that plasma protein binding controls and impairs the drug transfer from plasma to milk. Accordingly, sotalol and acebutolol, which are the less hydrophobic beta-adrenoceptor antagonists, are weakly bound to plasma proteins and have the highest milk-to-plasma concentration ratios. In contrast propranolol, which is a lipophilic drug but strongly bound to plasma proteins, has a milk to plasma concentration ratio less than 1.

In addition the binding parameters in milk correlate strongly with those in plasma (Fig. 2). Consequently, drugs which have high *NK* values for milk are strongly bound in plasma and curiously not concentrated in milk. Thus, these findings suggest that β blockers strongly bound in plasma should be used preferentially in lactating women.

In summary, the binding of beta-adrenoceptor antagonists in milk has been studied by equilibrium dialysis. The results of our investigation indicate that the major determinant of the transfer of this class of drugs into breastmilk is plasma binding. Thus simple *in vitro* studies including measurement of the serum binding, may aid in the selection of the treatment of hypertension for lactating women in order to limit adverse effects on the suckling infants. Further investigations involving other classes of drugs are necessary to draw general guidelines for most drugs.

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Verapamil modulates mutagenicity of antitumour acridines in bacteria and yeast

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A variety of antibiotics and synthetic DNA binding antitumour drugs are excluded from mammalian cells by an energy-dependent outward efflux mechanism. Resistance [1] may be acquired to drugs such as Adriamycin, amsacrine and vincristine by a mechanism, possibly resulting from gene amplification [2], whereby the outward efflux of drug is increased, leading to a reduction of its intracellular concentration [3, 4]. This mechanism, known as multiple drug resistance, can be at least partially inhibited by the calcium channel antagonist verapamil [5]. It is unlikely that this effect is mediated directly by changes in cellular calcium ion concentrations, and current evidence points to a direct effect of verapamil on the drug efflux mechanism [6].

We have previously investigated the bacterial mutagenicity of antitumour 9-anilinoacridine derivatives in microbial cells [7–9]. We have found that strongly basic 9-anilinoacridines (i.e. those with a high pK_a) have a lower mutagenicity in bacteria than would be expected on the basis of their DNA binding properties. Furthermore these drugs have a high efficiency of production of respiratory deficient ("petite") mutants in yeast. The inverse correlation between bacterial mutagenicity and yeast mitochondrial mutagenicity suggests that a drug transport mechanism may be operating on one hand to exclude positively charged drugs from bacteria and on the other to concentrate them in yeast mitochondria [9]. We show here that verapamil partially overcomes resistance in cultured mammalian cells to some of these positively charged acridine antitumour drugs, increases their mutagenicity in bacteria and decreases their efficiency in forming "petite" colonies in yeast.

The compounds studied (I–III, see Fig. 1 for structures) are related to the drug amsacrine, which was first synthesized by Cain and Atwell [10] and has now found widespread use in the treatment of acute leukaemia [11]. The parent compound I [4′-(9-acridinylamino)methanesulphonanilide], which lacks the 3′-methoxy group of amsacrine, was chosen because of its high DNA binding activity and its higher mutagenicity in microbial systems [7–9]. Murine P388 leukaemia cells and Adriamycin-resistant P388/ADR leukaemia cells were grown in culture (ref. 12 and B. C. Baguley and W. R. Wilson, *Eur. J. Cancer clin. Oncol.*, in press) and the degree of cross-resistance of the drug-resistant line was determined for (I), its 3-amino derivative (II), and its 3,6-diamino derivative (III) in continuous drug exposure growth inhibition assays (Table 1).

Cross-resistance to the parent compound (I) was 5-fold, similar to that shown for amsacrine itself (6-fold). However, cross-resistance to the strongly charged amino derivatives was considerably higher (160-fold and 77-fold respectively for compounds II and III). This finding is consistent with other results in this laboratory that a number of derivatives of amsacrine with amino or substituted amino groups on the acridine ring are much less effective against the P388/ADR cell line (B. C. Baguley, unpublished data). Proflavine (IV), a positively charged aminoacridine which lacks