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Comparison between immobilized artificial membrane (IAM) HPLC data and lipophilicity in *n*-octanol for quinolone antibacterial agents

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ABSTRACT

The membrane phospholipid affinity of ten quinolone antibacterial agents, including both acidic and zwitterionic compounds, was measured by HPLC on two different immobilized artificial membrane (IAM) stationary phases, namely IAM.PC.MG and IAM.PC.DD2; it is expressed as the logarithm of the retention factor measured with (or extrapolated to) 100% aqueous eluent at pH 7.0, log k_w^{IAM}.

Quinolones are a class of highly potent, orally active, broad-spectrum antibacterial agents. For these compounds, lipophilicity values in *n*-octanol found in the literature, either calculated or measured, are not consistent with each other and are too low to be compatible with their pharmacokinetic properties.

The log k_w^{IAM} values obtained in this study showed no relation with any of the lipophilicity values in the literature (clog *P*(a), clog *P*(b), MLP, log $D^{7.4}$). In contrast, they were collinear with a new lipophilicity scale we had previously obtained by an original ion-pair reversed-phase HPLC method set up to estimate the lipophilicity of the neutral forms, log P^N. Moreover, when comparing the retention of quinolones on IAM to the retention of structurally unrelated neutral compounds, we observed that they interact with phospholipids with the same affinity as neutral isolipophilic compounds.

The use of an eluent at pH 5.5, instead of pH 7.0, increased the retention on IAM not only for acidic, but also for zwitterionic congeners, indicating that phospholipid affinity is enhanced in the experimental conditions that depress the ionization of the acidic function, even when the ionization of the amino function increases simultaneously.

To gain an insight into the mechanism of quinolones/serum-protein interactions, we investigated about possible relationships between quinolones affinity data for serum proteins and IAM data. Quinolone affinity for both HSA and AGP was already demonstrated poorly related to *n*-octanol lipophilicity values, probably due to the occurrence of electrostatic interactions. Only poor relationships were found between IAM and HSA affinity data, whereas quite good relationships were found with AGP affinity data. However, IAM.PC.DD2 data correlated better than those on IAM.PC.MG with quinolone affinity for both serum-proteins, mainly due to the fact that IAM.PC.MG phase is scarcely discriminative for the compounds with the highest retention values.

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The results suggest that IAM retention can produce a lipophilicity scale that, unlike solvent/water partition coefficients, is consistent with the pharmacokinetic behaviour of zwitterionic quinolones.

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1. Introduction

The reliability of deducing drug partitioning into membranes from *n*-octanol/water partition coefficients ($\log P$) has been questioned for many years. Indeed, the interactions of ionised species with membranes is affected by ionic forces which are not accounted for by solvent/water partition coefficients. Therefore, partitioning systems including phospholipids as the lipophilic phase appear more appropriate to describe the partitioning of ionised drugs into membranes (Taillardat-Bertschinger et al., 2003).

In previous studies, we measured the partitioning of ionisable compounds (bases and acids) in phospholipids using immobilized artificial membrane HPLC (IAM-HPLC) (Amato et al., 2000; Barbato et al., 1996, 1997a, 1997b, 2004, 2005; Taillardat-Bertschinger et al., 2002). The results indicated that charged species partition in phospholipids better than expected on the basis of their partition in the *n*-octanol/water system. Indeed, charged analytes partition in *n*-octanol much poorly than isolipophilic neutral compounds. In contrast, the presence of a charged basic group on the analyte does not affect, or even promotes, partitioning in phospholipids, whereas the presence of a charged acidic group either does not affect or only produces a slight decrease of partitioning in phospholipids (much lower than that observed in *n*-octanol).

The different interaction capability of the two partitioning systems can be explained by the fact that only phospholipids support electrically charged moieties. Therefore, they can interact with charged analytes in such a way to produce an interaction scale modulated by the lipophilicity of the neutral form of solute, $\log P^N$, rather than by the lipophilicity of the ensemble of all charged and neutral forms existing at a given pH, $\log D^{pH}$. Moreover, the occurrence of additional interaction forces between phospholipids and charged analytes, such as attractive electrostatic interactions, has often been recognized (Taillardat-Bertschinger et al., 2003). This kind of forces are not encoded in the lipophilicity measures in *n*-octanol/water, because they cannot occur in isotropic biphasic systems, such as *n*-octanol/water, but only in anisotropic systems such as ordered phospholipid layers, i.e., when polar and apolar moieties are ordered in three-dimensional space, so offering different interaction capability depending on the side they are approached by the analyte.

To the best of our knowledge, to date, no comprehensive study has been performed about partitioning of zwitterionic ampholytes (zwitterions) into phospholipids. Zwitterions occur when $pK_{a_{acidic}} < pK_{a_{basic}}$ and can exist in solution as four different electrical forms with different lipophilicity values. Since no pH value of solution can assure the existence of the neutral form in such an extent to be considered predominant, it is impossible to measure the lipophilicity of the neutral form in *n*-octanol by the "shake-flask" technique (Leo et al., 1971).

As to the interaction between zwitterions and membranes, the role played by their charged moieties is far to be fully understood. Quinolones are a case in point: they are a class of highly potent, orally active, broad-spectrum antibacterial agents inhibiting the intracellular enzyme DNA gyrase. The class includes both acidic (the early quinolones) and zwitterionic (fluoroquinolones) compounds. The various lipophilicity values for zwitterionic quinolones reported in the literature, either determined in the *n*-octanol/water partition system or theoretically calculated, are very low and not consistent with each other. Nevertheless, quinolones are well absorbed when administered orally and massively penetrate into bacteria; since no active transport mechanism is involved (Merino et al., 1995), this implies that they can easily permeate gut wall and both bacterial cell wall and membrane by passive diffusion.

These quinolone pharmacokinetic aspects are inconsistent with their lipophilicity values; this may be due to the fact that both calculated and measured lipophilicity values reported in the literature take into account the simultaneous presence of the two opposite electrical charges on the molecule. Indeed, as above-mentioned, the partitioning degree of ionised species into membrane phospholipids is affected by electrical charges in a quite different extent than partitioning in *n*-octanol and it often depends just on the lipophilicity of the neutral species, $\log P^N$ (Amato et al., 2000; Barbato et al., 1997a, 1997b, 2004, 2005; Taillardat-Bertschinger et al., 2003). Therefore, we decided to achieve direct measures of phospholipid affinity for ten quinolones, including both acidic (nalidixic acid, oxolinic acid, cinoxacin, and flumequine) and zwitterionic congeners (pipemidic acid, ciprofloxacin, rufloxacin, piromidic acid, ofloxacin, and norfloxacin) (Scheme 1), by measuring their retention on two different phospholipid stationary phases (namely, IAM.PC.MG and IAM.PC.DD2).

The aim of this study has been to investigate on possible relationships between IAM retention data and various lipophilicity scales, both theoretically calculated and experimentally measured, including a lipophilicity scale we previously determined by ion-pair reversed phase high performance liquid chromatography and assumed to mirror the lipophilicity of the neutral forms of the analytes (Barbato et al., 2007). The variation of partition in phospholipids as function of the pH of the eluent was also investigated. Finally, as IAM data encodes not only lipophilic, but also electrostatic intermolecular recognition forces (Taillardat-Bertschinger et al., 2003), we investigated about possible relationships between quinolone affinity data for serum proteins (Barbato et al., 2007) and IAM retention values to gain an insight into the mechanism of quinolones/serum-proteins interactions.

2. Materials and methods

2.1. Materials

All samples were obtained from commercial source. All chemicals were of HPLC grade and used without further purification.

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