Potentiometric determination of the dissociation constants of epirubicin HCl and irinotecan HCl

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DOI: 10.1070/MC2006v016n02ABEH002234

Dissociation constants and relative abundances of ionised and non-ionised forms of epirubicin HCl and irinotecan HCl were determined potentiometrically.

Because most drugs are absorbed by passive diffusion of the non-ionised moiety, the extent to which a drug is in its ionised and non-ionised forms at a certain pH is of great importance for the behaviour of drugs in penetrating to different body compartments.[1] The dissociation constants and relative abundances of non-ionised forms of epirubicin HCl and irinotecan HCl were determined potentiometrically around the range of the concentrations given to the patients clinically to evaluate the relation of their pK_a and pharmacokinetics, as well as their dissociation and precipitation in blood.[2–4]

Epirubicin HCl and irinotecan HCl are widely used in clinical cancer therapy[3,4] and administered directly to blood in 0.9% NaCl or 5% dextrose solutions through long period infusions.[5] Considering that these drugs may be used in combination because of their effects on similar organs, the chemical interaction of these drugs in an infusion solution was studied previously.[6] Since most of the drugs are weak acids or bases, their behaviour in blood and infusion solutions largely depends on their ionization constants (pK_a), the final pH of the infusion solution and the concentration of the non-ionised form of the drug in this solution.[7] Especially, for understanding and quantifying the reaction rates, biological activity, biological uptake, biological transport and environmental fate,[8] dissociation constant (*i.e.*, pK_a) is a key parameter.[9]

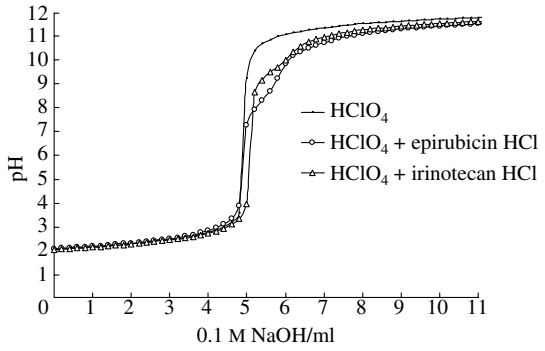

Figure 1 Potentiometric titration curves of $HClO_4$, irinotecan HCl and epirubicin HCl.

The experimental procedure is as follows. Solution (1): 5 ml, 0.1 M $HClO_4$ solution; solution (2): 5 ml 0.1 M $HClO_4$ solution + 20 ml 5×10^{-3} M epirubicin HCl sample, which was freshly prepared by dissolving in a 0.9% NaCl solution; solution (3): 5 ml 0.1 M $HClO_4$ solution + 10 ml 5×10^{-3} M irinotecan HCl sample, which was freshly prepared by dissolving in water (because 10 ml of a 0.9% NaCl solution was not enough volumetrically to dissolve this concentration of irinotecan HCl). The ionic strengths (I) of solutions (1), (2) and (3) were adjusted with 1 M NaCl, while completing to some volume, so that the ionic strength and the NaCl concentration of 0.9% NaCl infusion solution were imitated. Each sample mixture was titrated potentiometrically with a 0.1 M NaOH solution in the dark at room temperature (Figure 1). Using the Irving–Rossotti method,[10] $\bar{n}_A$ values were calculated from the titration curves of $HClO_4$, plotted as a function of pH, *i.e.*, $\bar{n}_A = f(\mathrm{pH})$. From the graph, log K values were calculated.

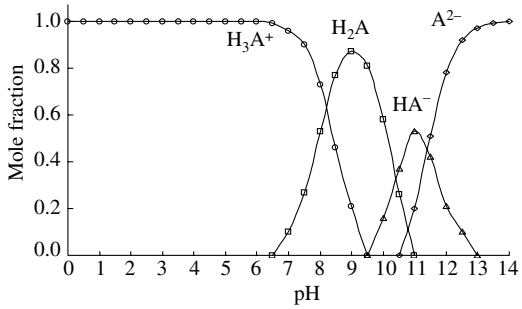

Figure 2 Relative abundances of epirubicin species *versus* pH.

Using the dissociation constant (acid constant) values (Table 1), the variations of the relative abundances of the ionic species derived from epirubicin HCl (non-ionised form is denoted as H_2A) and irinotecan HCl (non-ionised form denoted as A) were plotted against pH (Figures 2 and 3).

When the protonations of the groups in the molecule are considered, it is deduced that the log K_3 (pK_{a1}) value of 8.50 is due to the protonation of the amine; the log K_2 (pK_{a2}) value of 10.75 and the log K_1 (pK_{a3}) value of 11.33 are due to the protonation of phenolic OH groups in the molecule. For the irinotecan HCl molecule, log K_1 (pK_a) value of 8.79 is also deduced to belong to the protonation of the tertiary amine inside the molecule. The prediction of the pKa values using pK_a/PALLAS program[11] is satisfactory and consistent with the experimental data. This is the first report on the determination of the pK_a of irinotecan HCl and epirubicin HCl in water or in infusion solutions.

Since these drugs are administered directly to blood (pH 7.4), and as seen from Figures 2 and 3, most of the drugs exist in ionised forms in blood. Thus, the transfer through membranes will be slow and penetration to the tissues will be less than that of a non-ionised drug. As the non-ionised form (which exists in smaller percent) passes through the membranes, equilibrium moves in favour of the non-ionised form in blood. Since the non-ionised form has a poor solubility, it arises as a better situation for these drugs to exist in lesser extent in non-ionised form in blood. The second and third ionised forms of epirubicin do not exist at physiological pH, as can be seen in Figure 2. When the pharmacokinetic parameters of both drugs are considered in regard to pK_a constants, the good penetration to

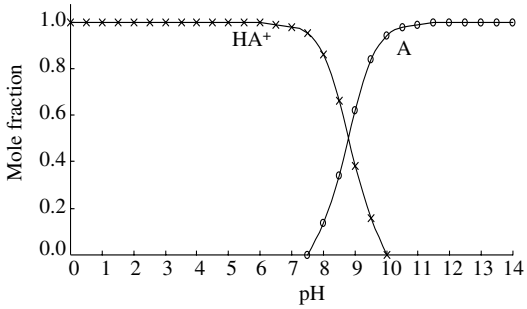

Figure 3 Relative abundances of irinotecan species *versus* pH.

Table 1 Predicted and experimental pK_a values of irinotecan HCl and epirubicin HCl.

	PKALC[a]	pK_a[b] experimental (n = 5)
Epirubicin HCl	pK_{a1} = 8.57 pK_{a2} = 9.26 pK_{a3} = 11.23	pK_{a1} = 8.5 (±0.20) pK_{a2} = 10.75 (±0.17) pK_{a3} = 11.33 (±0.19)
Irinotecan HCl	pK_a = 8.93	pK_a = 8.79 (±0.18)

[a]pK_a values predicted by Pallas/PKalc software package. [b]pK_a value of USP Standard of drugs in 0.9% NaCl infusion solution at 25 °C, I = 0.154 M. Values in parantheses are standard deviations.

body compartments[1] and long time effects seem to arise merely because of protein binding, passing through the porous membranes of vessels and binding of these drugs to tissues.

We are grateful to Professor Emre Dölen, Research Fund of Marmara University, Professor Serdar Turhal, Dr. Mahmut Gümüş and Nurse Ayla Karaman, Pharmacia-Italy and Pharmacia-Turkey, Aventis Pharma-Turkey, Aventis Pharma-France.

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Received: 30th August 2005; Com. 05/2571