SHORT COMMUNICATIONS

In vitro Study of the Effect of Paracetamol on Drug-Protein Interactions

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Association constants for the binding of six drugs to serum albumin have been studied in the absence and presence of paracetamol using ultraviolet absorption spectroscopic technique. Paracetamol is found to influence the binding behaviour of other drugs by non-competitive interference involving structural changes in the albumin molecule. Significant increase in the concentration of free drug has been observed for metronidazole and promethazine hydrochloride in the presence of paracetamol. Ratio of association constants in the presence and absence of paracetamol can be used as a guide to the modified design of dosage forms in the presence of paracetamol.

The nature and magnitude of drug-protein binding has important pharmacokinetic and pharmacodynamic implications, because it is the unbound moiety that readily diffuses across biological memberanes, reaches the receptor site to produce pharmacological effect, and is most readily available for elimination from the body[1-2]. The simultaneous administration of two or more strongly bound drugs can compete with one another for the binding sites on albumin and so result in displacement interactions[3-5]. Paracetamol, a very commonly used analgesic and antipyretic drug, is frequently prescribed along with other medicines. Although paracetamol is not strongly bound at therapeutic concentrations, it can still affect the protein binding behaviour of other drugs either by blocking an active site or by causing conformational changes in the protein molecule. Thus, the presence of paracetamol can significantly alter the pharmacological response of other drugs by altering the concentration of free drug in plasma. Present work was, therefore, undertaken.

Paracetamol, metronidazole, tinidazole, furazolidone, promethazine hydrochloride, metoclopramide and caffeine were obtained as gifts from various manufacturers. Serum albumin bovine (BSA) was purchased from Sigma Chemical Company, St. Louis, USA. All other reagents were of analytical grade.

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All solutions were prepared in 0.1 M phosphate buffer of pH 7.4 containing 0.15 M NaCl. Protein binding was studied using ultraviolet absorption spectroscopic technique. Ultraviolet absorption spectra were determined in the range 200-400 nm on a Hitachi 330 UV-VIS spectrophotometer. For single drugs, 1.5 ml of 25 μM BSA solution was taken in sample cell and 1.5 ml phosphate buffer in reference cell. Increasing amounts of 525 μM drug solution was then added in both the cells. In the sample cell, BSA concentration was kept constant (25 μM) by adding same volume of 50 μM BSA. In the reference cell, increasing amount of drug and buffer were added so as to keep drug concentration exactly same in the sample and reference cells. The final drug concentration was close to 150 μM corresponding to drug:protein ratio of 6:1 Absorbance was determined at 280 nm after each addition of drug. Shift in absorbance, ΔA was obtained in each case as the difference between the absorbance of BSA in the absence and presence of drug. In another series of experiments, drug-protein interaction was studied in the same way but in the presence of fixed concentration of paracetamol (50 μM).

In the case of competitive binding involving more than one ligand, the interpretation of the data is easier and more meaningful when the stoichiometry of the interaction is assumed to be 1:1 so that the calculated association constants represent the weighted average of several binding sites[6]. Since the total concentration of ligand (drug) is much greater than the total acceptor (protein) concentration, the concentration of bound drug does not contribute appreciably to the total concentration of drug. Under such conditions, the free drug concentration can be essentially equated with the total drug concentration and the maximum change in absorbance, ΔA_{max} can be obtained from the intercept at $1/D_T=0$ of the conventional double reciprocal plots ($1/\Delta A$ versus $1/D_T$, where D_T is the total concentration of drug added)[7]. $1/\Delta A$ versus $1/D_T$ plots for one representative sample, tinidazole in the absence and presence of paracetamol, are shown in fig.1.

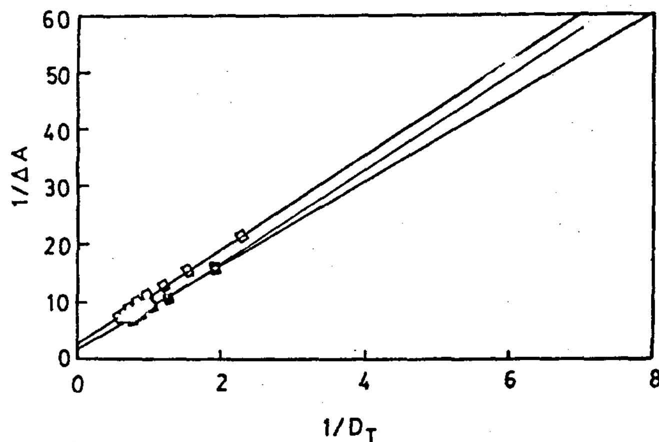

Fig. 1: $1/\Delta A$ versus $1/D_T$ plots for Tinidazole.

ΔA is the difference in the absorbance of BSA in the absence and presence of drug and D_T is the total drug concentration. (-■-) represents tinidazole while (-□-) represents tinidazole in the presence of paracetamol.

Fraction of occupied sites on the protein molecule, $\theta=\Delta A/\Delta A_{max}$. Since a single binding site is assumed, concentration of bound drug=concentration of bound protein=θP_T, concentration of free drug, $D_F=D_T-D_B=D_T-\theta P_T$ and concentration of free protein, $P_F=P_T-P_B=P_T-\theta P_T$,

Where P_T and D_T are the total protein and drug concentrations and P_B and D_B are the bound protein and drug concentrations, respectively. Figs. 2 and 3 show the concentration of free drug (D_F) in the absence and presence of paracetamol as a function of the total drug added (D_T).

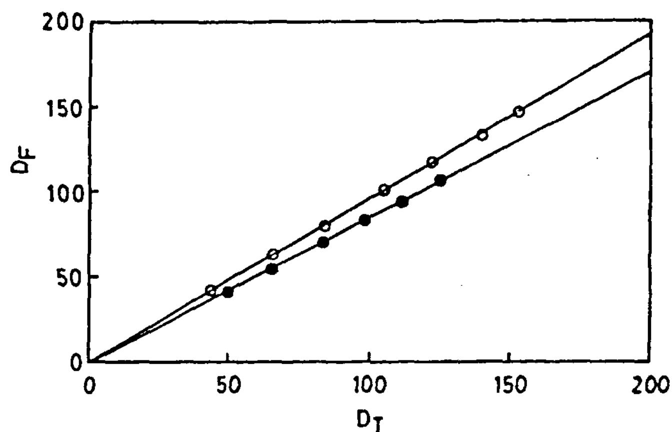

Fig. 2: D_F versus D_T plots for Metronidazole.

D_F is the concentration of free drug and D_T is theTotal drug concentration. (-●-) represents motronidazole while (-O-) represents motronidazole in the presence of paracetamol

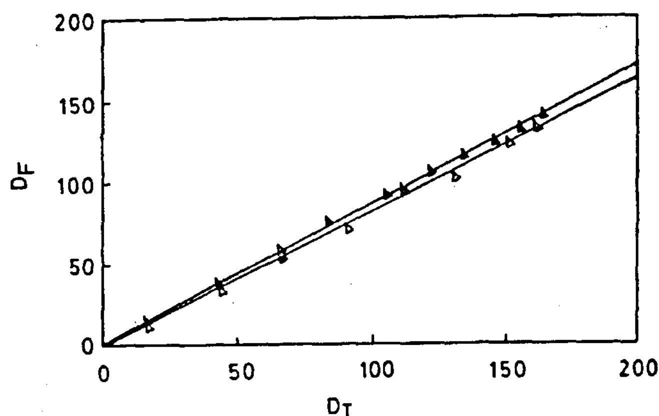

Fig. 3: D_F versus D_T plots for metoclopramide.

D_F is the concentration of free drug and D_T is theTotal drug *concentration.* (-▲-) *represents metronidazole while* (-Δ-) represents metronidazole in the presence of paracetamol.

Moles of drug bound per mole protein, $r=D_B/P_T=\theta P_T/P_T=\theta$

Thus association constant, K for the binding equilibrium could be obtained from a plot of $1/\theta$ versus $1/D_F$. $1/\theta$ versus $1/D_F$ plots for tinidazole in the absence and presence of paracetamol are shown in fig. 4.

Addition of drug to BSA resulted in a decrease in absorbance in most cases. However, no noticeable shift in wave length corresponding to the absorption maxima was observed. Association constants, K, for six drug samples used in the present work, in the absence and

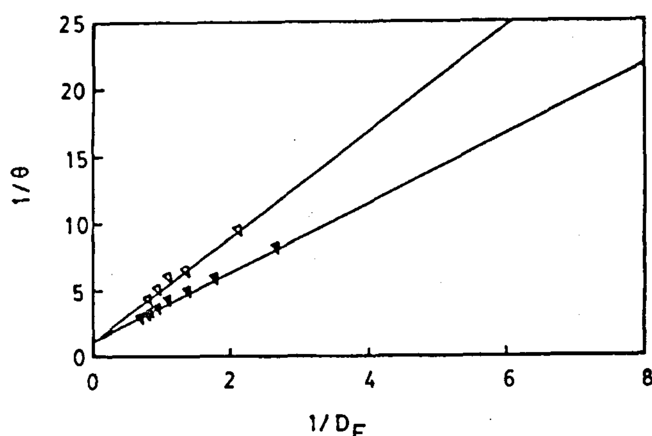

Fig. 4: 1/θ versus 1/D_F plots for tinidazole in the absence and presence of paracetamol.

θ(=ΔA/ΔA_{max}) is the fraction of occupied sites on the protein molecule and D_F is the free drug concentration. (-▼-) represents tinidazole while (-∇-) represents tinidazole in the presence of paracetamol.

presence of paracetamol, are given in Table 1. Values are of the order of 10^3. It is seen that the magnitude of association constants is lower than the values reported in the literature [8-10] for some of the samples. Since a single binding site is assumed, the association constant values represent average of values for high and low affinity sites and are thus lower[6]. Moreover, most of the literature values have been obtained using fluorescence spectroscopic technique. Fluorescence quenching measurements are only sensitive to perturbations in the tryptophan residue, which is the high affinity site. Secondary binding at sites of lower affinity, remote from the fluorescent moiety are not detected. Association constants calculated from absorption difference spectroscopic data, on the other hand, are more responsive to general alterations in the drug molecule environment and thus represent an average of several binding sites. Similar findings have also been reported by other workers[6,11]. However, in the present context only relative values of association constants in the presence and absence of paracetamol are significant.

It is seen that the presence of paracetamol significantly influences the binding behaviour of different drugs. Both increase and decrease in association constants of drugs have been observed in the presence of paracetamol, as compared to drug alone. However, since paracetamol is not strongly bound to serum albumin[12], it is unlikely to cause competitive displacement of other drugs from their bindings sites. Both increase as well as decrease in association constants in the presence of paracetamol can be interpreted as non-competitive interference. It may be inferred that either paracetamol blocks the active drug-binding sites or simultaneous binding of paracetamol and other drugs produces significant structural changes in the albumin molecule resulting in alteration in the nature and number of binding sites. Such non-competitive interference can cause changes in the concentration of free drug in plasma and hence the pharmacological response.

Reference to Table 1 shows that association constants of caffeine, metronidazole and promethazine hydrochloride decrease whereas those of tinidazole, furazolidone and metoclopramide increase in the presence of paracetamol. The decrease/increase in the associa-

TABLE 1: ASSOCIATION CONSTANTS OF VARIOUS DRUGS IN THE ABSENCE AND PRESENCE OF PARACETAMOL

Sample	**Association constant (K) x 10^{-3}**		**K_{ratio}**
	in the absence of paracetamol	**in the presence of paracetamol**	
Caffeine	1.323	0.565	0.427
Metronidazole	5.814	1.032	0.177
Promethazine hydrochloride	18.058	2.474	0.137
Tinidazole	2.777	4.353	1.568
Furazolidone	1.192	2.887	2.422
Metclopramide	5.045	8.204	1.626

K_{ratio} = Association constant in the presence of paracetamol/Association constant in the Absence of paracetamol.

TABLE 2: CONCENTRATION OF FREE DRUG IN THE PRESENCE AND ABSENCE OF PARACETAMOL

Drug	Concentration of free drug (D_F)(μM)*	
	No paracetamol	paracetamol
Caffeine	94.05	97.35
Metronidazole	85.37	95.56
Promethazine hydrochloride	85.98	95.38
Tinidazole	91.09	89.10
Furazolidone	94.14	91.69
Metclopramide	85.10	82.05

*Concentration of free drug has been reported at a total drug concentration (D_T) of 100 μM.

tion constant of a drug in the presence of paracetamol increases/decreases the concentration of free drug in plasma. Increase in the concentration of free drug enhances the pharmacological effect and may produce toxic reaction whereas decrease in the concentration of free drug may not produce the desired therapeutic effect. Plots of the concentration of free drug, D_F against the total drug added, D_T also shown that in the presence of paracetamol, the concentration of free drug increases in the case of caffeine, metronidazole and promethazine hydrochloride and decreases in the case of tinidazole, furazolidone and metoclopramide. The D_F versus D_T plots for metronidazole and metoclopramide are shown in figs. 2 and 3. Table 2 shows the concentration of free drug in the presence and absence of paracetamol at a total drug concentration of 100 μM. (100 μM drug corresponds to about 15 mg/l and falls in the therapeutic range, 10-20 mg/l[13]). Therefore, to have the same pharmacological effect, the doses of these drugs have to be modified in the presence of paracetamol.

Once the interference of paracetamol in the protein binding of a drug is established, one can anticipate the need for an adjustment in dosage in the presence of paracetamol. The relative ability of paracetamol to interfere in the binding of other drugs can be quantitatively determined from K_{ratio}, the ratio of association constants in the presence and absence of paracetamol (Table 1). K_{ratio} can, therefore, be a guide to the modified design of dosage forms in the presence of paracetamol.

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