Importance of Radiochemical Purity of Radiolabeled Drugs Used for Determining Plasma Protein Binding of Drugs

Keyphrases □ Binding, plasma protein—effect of radiochemical impurity of radiolabeled drugs □ Drugs, radiolabeled—effect of radiochemical impurity on determination of plasma protein binding of drugs □ Impurity, radiochemical—of radiolabeled drugs, effect on determination of plasma protein binding of drugs

To the Editor:

Plasma protein binding of drugs has been the subject of intensive study for many years. It is an important pharmacological parameter, since it frequently affects drug distribution and elimination (1–3) and duration and intensity of pharmacological effects of drugs (4–6). A number of factors are known to affect the determination of drug protein binding, including the use of vacuum container[1] blood collection tubes (7, 8), some polyvinyl intravenous tubing (7, 9), and the administration of heparin (used therapeutically or as heparin lock) (9, 10). All of these factors result in diminished drug binding. An additional factor that has not received sufficient attention, although it has been stressed (11), is the importance of radiochemical purity of the radiolabeled drugs used in the determination of drug protein binding.

Equilibrium dialysis has become the most commonly used method for determining drug protein binding in clinical pharmacokinetic studies. Tracer amounts of radiolabeled drugs are added on either side of the dialysis membrane, usually on the buffer side. After dialysis has reached equilibrium, the unbound or free fraction of the drug (f_f) is usually calculated as the ratio of the radioactivity determined in the buffer phase to that determined in the plasma phase, correcting for volume differences, if any, between the two phases subjected to liquid scintillation counting, as follows:

$$f_f = \frac{R_B \times V_P}{R_P \times V_B} \qquad \text{(Eq. 1)}$$

where R_B and R_P represent the radioactivities determined in the buffer and plasma phases, respectively, and V_P and V_B represent the volumes of the plasma and buffer phases counted, respectively.

These calculations assume that the radiolabeled drug used is absolutely void of any radiochemical impurities. However, this is usually not the case unless the radiolabel is purified before use, since most commercially available radiolabeled drugs contain unknown, although small, amounts of radiochemical impurities. These radiochemical impurities containing the radioactive isotope (usually carbon 14 or tritium) may be a starting material or intermediary compounds in the chemical synthesis of the radiolabeled drug, or breakdown products of the drug.

[1] Vacutainer.

To evaluate the effects of varying degrees of radiochemical impurities present in the dialysis on the determination of plasma protein binding of drugs, Eq. 1 has to be modified. The observed free fraction (f_f') can be calculated for varying combinations of the true or actual free fraction (f_f) and the fraction of impurities in the radiolabel used (f_i) assuming that V_P equals V_B, using the expression:

$$f_f' = \frac{[R_T - (R_T \times f_i)]\, f_f + \left(\frac{R_T \times f_i}{2}\right)}{[R_T - (R_T \times f_i)] + \left(\frac{R_T \times f_i}{2}\right)} \qquad \text{(Eq. 2)}$$

where the numerator represents R_B and the denominator represents R_P in Eq. 1 and R_T represents the total radioactivity added (consisting of both the drug of interest and the impurities); it is assumed that the radiochemical impurities are not bound to plasma proteins and distribute evenly in the plasma and buffer phases. The former terms in the numerator and denominator in Eq. 2 represent the actual free fraction, while the sum of the latter terms in the numerator and denominator represents the radioactivity due to radiochemical impurities.

Simulations were carried out using Eq. 2 to illustrate the effects of varying degrees of radiochemical impurities in the radiolabel used on the determination of plasma protein binding of drugs. Figure 1 shows simulations of the observed free fraction *versus* the actual free fraction, when the impurities in the radiolabel vary from 2.5–10% of the total radioactivity added. It can be seen that increasing the percentage of impurities in the radiolabel results in an

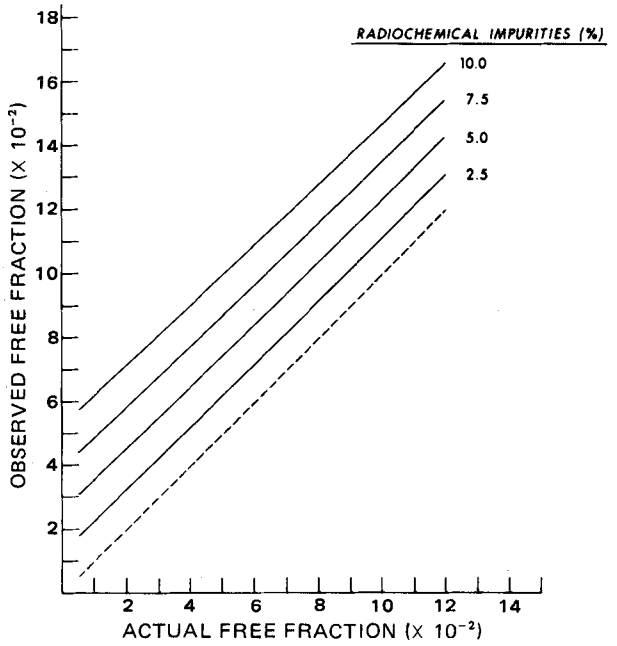

Figure 1—*Effects of varying degrees of radiochemical impurities present in dialysis on the determination of plasma protein binding of drugs.*

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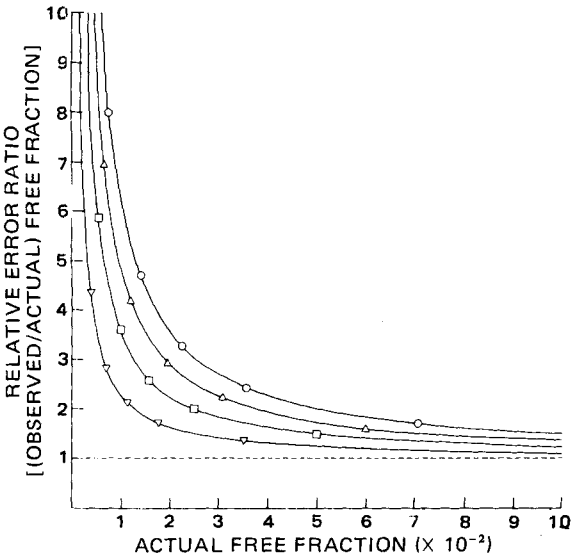

Figure 2—*Effects of varying degrees of radiochemical impurities present in dialysis on the relative error involved in the determination of plasma protein binding of drugs. Key:* ○, *10.0;* △, *7.5;* □, *5.0; and* ▽, *2.5% radiochemical impurities.*

upward shift of the linear relationship between the observed *versus* the actual free fraction.

However, the relative error on the determination of drug protein binding caused by the radiochemical impurities varies markedly depending on the actual free fraction; the lower the actual free fraction the greater the relative error and vice versa. This relative error can be expressed as the ratio of the observed to actual free fraction, which indicates how many times higher the observed free fraction is than the actual free fraction.

Figure 2 shows a plot of this relative error ratio *versus* the actual free fraction. It has an expected hyperbolic form for any given degree of radiochemical impurities present because the independent variable is in the denominator of the dependent variable. It can be seen from Fig. 2 that the observed free fraction will be ~2.3–6.3 times higher than an actual free fraction of 0.01 when radiochemical impurities vary from 2.5–10%, while the observed free fraction is only ~1.1–1.5 times higher than an actual free fraction of 0.1 with the same range of radiochemical impurities present.

The use of a radiolabel containing radiochemical impurities in dialysis for the determination of protein binding in clinical pharmacokinetic studies will not only result in a wrong value, but it will make all comparisons of binding data difficult. Such comparisons are frequently of interest, *e.g.*, when evaluating the effects of other drugs or disease states on the protein binding of drugs or when comparing drug binding in different groups of age or sex. This effect can be illustrated by two groups of drug binding data with average actual free fractions of 0.01 and 0.02. When a radiolabel with 5% radiochemical impurities is used, this 100% difference in the actual free fractions will reduce to a 28% difference in the free fractions, *i.e.*, the observed free fractions will be 0.035 and 0.045. It is likely that a true statistically significant difference among various groups of binding data may therefore become obscured. Because of the significance of drug protein binding in clinical pharmacology, it is suggested that radiolabels used for the determinations of plasma protein binding of drugs be purified prior to dialysis, *e.g.*, by high-pressure liquid chromatography, and that the stability of the radiolabel be verified postdialysis in both buffer and plasma phases.

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Determination of Renal Clearances Using Arterial and Venous Plasma: Procainamide in Rabbits

Keyphrases □ Procainamide—renal clearance, determination using timed-interval method, rabbits □ Renal clearance—determination of procainamide using arterial and venous plasma, rabbits □ Pharmacokinetics—determination of renal clearance of procainamide using arterial and venous plasma, rabbits

To the Editor:

The timed-interval method is commonly employed in pharmacokinetic studies to determine the renal clearance (Cl_r) of a drug compound:

$$Cl_r = \frac{X_{t_1-t_2}}{AUC_{t_1-t_2}} \qquad \text{(Eq. 1)}$$

where $X_{t_1-t_2}$ and $AUC_{t_1-t_2}$ are the amount of the intact drug excreted and the area under the plasma concentration curve between times t_1 and t_2, respectively. To date, the potential effect of the source of plasma data, either arterial or venous, on the estimation of clearance using Eq. 1 has rarely been evaluated. The arterial and venous differences of six compounds were recently reported (1). The present

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