Part II

Physiological Variations in Drug-Protein Binding

11

INTERINDIVIDUAL DIFFERENCES IN DRUG-PROTEIN BINDING

Folke Sjöqvist and Yuichi Koike

INTRODUCTION

Individualization of drug dosage is a fundamental principle in clinical pharmacology and therapeutics. Ideally, this principle is practiced by titrating the dosage schedule against the clinical effects. This is greatly facilitated by the availability of sharp pharmacological or biochemical endpoints of drug action. However, the effects of many drugs are not easily discernible in the individual patient, and it is then virtually impossible to evaluate the dose-response curve. Prophylactic therapy and drug therapy in neuropsychiatry are particularly difficult areas in this respect. In addition, many antiepileptic, antidepressant, and neuroleptic drugs are characterized by pronounced interindividual variability in pharmacokinetics, resulting in a wide scatter of drug plasma levels at fixed dosage schedules.

The concept of therapeutic drug monitoring is based on the premise that the clinical effects are better correlated to drug plasma levels than to the prescribed dosage. An "abnormal" drug concentration should thus serve as an early warning signal to the clinician to modify the dosage schedule. The utilization of therapeutic drug monitoring must be based on results from controlled clinical studies which evaluate the relationship between drug concentrations in plasma and clinical outcome. The validity of the data obtained will depend on the usual criteria of controlled clinical investigation, such as study

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design, patient selection, sample size, and most of all, the methods used for evaluating therapeutic and toxic effects. Too many of these studies can be described as an unhappy marriage between sophisticated kinetics and unsophisticated clinical investigation. Hence the list of drugs suitable for therapeutic drug monitoring is still very short.

A major determinant of the interindividual variability in kinetics is differences between individuals in drug clearance due to pathophysiological, genetic, and environmental factors. It is well established that this source of variability by far outweighs the clinical importance of interindividual differences in plasma protein binding, at least in metabolically healthy individuals without compromised kidney and liver function (Sjöqvist, Orme, and Borgå 1980b). During the first two decades of therapeutic drug monitoring, measurement of unbound drug in plasma was considered to be a sophisticated last step to be applied on very strict indications. Recent advances in technology permit more liberal indications and some authors now advocate "routine" measurements of unbound concentrations of certain drugs such as antiepileptics. This idea is based on two assumtions. Firstly, interindividual differences in drug binding are said to be clinically important, and largely unpredictable from the patient's clinical condition, the concentrations of binding proteins and effects of concomitant drug therapy. Secondly, it is taken for granted that drug effects correlate better to unbound concentrations than to total drug in plasma. Let us examine the evidence by looking at diphenylhydantoin (DPH) and tricyclic antidepressants (TCA) which represent two classes of drugs, acidic and basic, with different binding characteristics.

DIPHENYLHYDANTOIN—AN ACIDIC DRUG

Very early in our initial studies of DPH, we were impressed by the small (less than two-fold) interindividual differences in binding (Lunde et al. 1970) in the absence of complicating factors such as displacement interactions (loc. cit.), hyperbilirubinemia (Rane et al. 1971), or uremia (Reidenberg et al. 1971). This general impression was confirmed in an extended study of 63 epileptic patients, where we found an unbound fraction of 7.1 ± 1.0 percent with a range of 4.9 percent to 10.2 percent (Fig. 11-1). We also found that individual phenytoin binding was reproducible when the determination was repeated several weeks later. A strong correlation was found between the total and the unbound drug concentration in plasma ($r = 0.97$) and between salivary and plasma concentrations of DPH ($r = 0.83$).

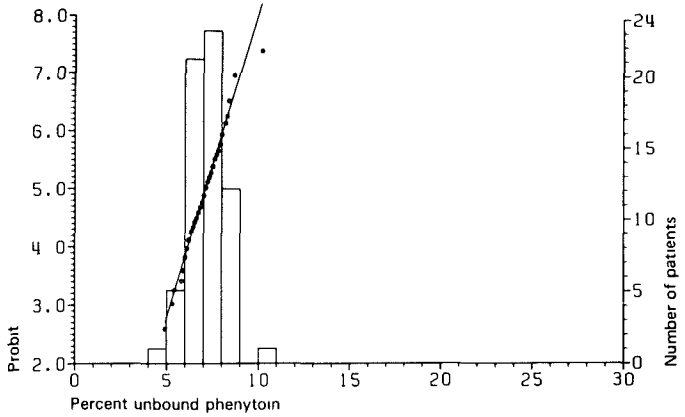

FIGURE 11-1
Probit plot and histogram of percent unbound phenytoin in plasma samples from 63 patients with grand mal and mixed epilepsy. Source: Barth et al. (1976). Reprinted with permission.

At the time, our findings were at variance with other authors who had reported considerably greater variation between individuals, most notably Booker and Darcey (1973). There are now three other studies, summarized in Table 11.1, which also have failed to demonstrate more than two-fold interindividual variability in DPH-binding in metabolically healthy epileptic patients or volunteers. In the study of Peterson et al. (1982), most of the outliers with a relatively high free fraction were either treated concomitantly with potential displacers or suffered from diseases known to affect acidic drug binding.

The patient material in the study of Booker and Darcey (1973) is not described in detail with respect to other drugs used, kidney and liver function, and some questions regarding the validity of the technique used for measuring unbound DPH (protein leakage?). Nevertheless, the authors reported on the basis of qualitative judgments that signs of toxicity were better related to free than to total DPH levels and only occurred at free concentrations above 1.8 μg/ml. Similar data have recently been reported by DeMonaco and Lawless (1983), who measured unbound DPH with a commercial method. The authors state that toxicity in 9 out of 31 patients occurred at unbound concentrations above 2 μg/ml and that only 3 of these patients had total concentrations above 20 μg/ml. Unfortunately, this study suffers from the many shortcomings of an uncontrolled clinical investigation. In fact, no physician coauthored the paper and nothing is said about the methods used for assessing symptoms in the patients.

From these considerations, it would appear that "routine" estimation of free DPH in plasma is unnecessary and should be

reserved for patients where alteration in binding is likely to occur—e.g., renal or hepatic disease, simultaneous treatment with valproic acid or other displacers, and when the clinical response deviates from that expected relative to total DPH in plasma.

Needless to say, controlled clinical investigations of the relationship between unbound DPH concentrations and seizure control should be encouraged. Such studies should benefit from the simplified and validated methods that now are available for measuring unbound DPH in plasma (Fig. 11-2). The crucial question that arises is whether our present methods for assessing antiepileptic drug action will be sensitive enough for this venture.

TABLE 11.1
Interindividual Differences in Plasma Protein Binding of Phenytoin

Method	n	Subject Characterization	Percent Free Mean (Range)	Reference
Ultrafiltration at room temperature. Specified conditions	63	Patients with grand mal or mixed epilepsy, 24-79 years old, without biochemical evidence of liver and kidney dysfunction. Not treated with known displacers (with one exception)	7.1 ± 1.0 (SD) (4.9-10.2)	Barth et al. (1976)
Equilibrium dialysis at 37°C. Specified conditions	39	Normal healthy adults, 18-54 years old, not treated with known displacers	13.6 (11.1-15.5)	Yacobi et al. (1977)
Equilibrium dialysis at 37°C. Unspecified conditions	100	Patients with epilepsy, 5-90 years old, on other antiepileptic drugs. Five had abnormal kidney or liver function	12.3 (median) (9.7-24.7) 80% within 9.7-14.5. Twelve patients with low binding were on valproic acid (7), warfarin (2), and other displacers (3). Four other low binders had alcoholic liver disease	Peterson et al. (1982)
Equilibrium dialysis at 37°C. Specified conditions	56	Patients with epilepsy. 13-72 years old, without biochemical evidence of renal or hepatic dysfunction. Four were on valproic acid	14.5 ± 1.2 (SD) (12.3-17.7) Valproate patients 17	Rimmer et al. (1984)

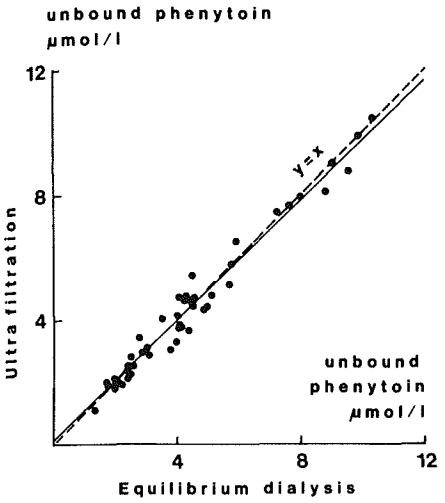

FIGURE 11-2
Relationship between the unbound phenytoin concentrations determined by ultrafiltration and equilibrium dialysis in 50 samples including uremic patient samples. The slope was 0.97, the intercept 0.06, and r = 0.99 ($p < 0.001$). Ultrafiltration was performed with Emit Free Level$^{T.M.}$ Filter I.
Source: Koike et al. (1985).

TRICYCLIC ANTIDEPRESSANTS AND OTHER BASIC AMINES

Our experience with basic amines (tricyclic antidepressants, alprenolol, chlorpromazine) is similar to our experience with phenytoin (Sjöqvist, Bertilsson, and Åsberg 1980a; Piafsky and Borgå 1977; Piafsky et al. 1978). Usually, two-fold interindividual differences have been found in the free fraction. Occasional outliers have often had suspected or documented changes in the concentration of α_1-acid glycoproteins which bind these drugs (Fremstad, Bergerud, and Haffner 1976) and are elevated in various infectious diseases such as Chron's disease and rheumatoid arthritis, where base binding is increased (Piafsky et al. 1978). As an example, a depressed patient with an unusually low unbound fraction of imipramine described by Glassman, Hurwix, and Perel (1973) also suffered from rheumatoid arthritis (personal communication from Dr. Glassman). Table 11.2 is a compilation of previous and recent studies of interindividual differences in binding of tricyclic antidepressants. The most recent study by Pike and Skuterud (1982) demonstrated a two-fold inter-

TABLE 11.2
Interindividual Differences in Plasma Protein Binding of Antidepressant Drugs

Drug	Technique	n	Percent Free Mean (Range)	Characterization of Subjects (Reference)
Desmethyl-imipramine	Ultrafiltration, room temperature	41	9.5 ± 1.4 (SE)	Healthy blood donors (Borgå et al. 1969) Only one outlier with 5% free (Sjöqvist et al. 1969)
Desmethyl-imipramine	CSF/plasma ratio	15	15 (11-17)	Patients treated with imipramine (Muscettola et al. 1978)
Imipramine	Equilibrium dialysis at 37°C for 17 hr	23	7.9 (6.1-11.1)	Healthy subjects with documented medical history. Albumin and α_1-acid glycoprotein measured (Piafsky and Borgå 1977)
Imipramine	Equilibrium dialysis at 37°C for 36-48 hr	26	5.4-23	Depressed patients, concomitant drugs and diseases not specified. 23 patients were within a twofold range of 8-18% (Glassman et al. 1973)
Imipramine	CSF/plasma ratio	15	11 (7-12)	Patients treated with imipramine (Muscettola et al. 1978)
Nortriptyline	Equilibrium dialysis	34	"Binding ratio measured"	Healthy twins not treated with other drugs. Less than twofold interindividual variability (Alexanderson and Borgå 1972)
Nortriptyline	CSF/plasma ratio	13	7.0 ± 1.8 (SD) (4.7-11.5)	Patients treated with nortriptyline (Kragh-Sørensen et al. 1976)
Nortriptyline	Equilibrium dialysis at 37°C for 3 hr	35	5.4-11.3	20 depressed patients. 15 intoxicated patients. Plasma proteins were measured (Pike and Skuterud 1982)
Amitriptyline	Equilibrium dialysis at 37°C for 3 hr	52	3.5-7.4	Depressed patients. Plasma proteins were measured (Pike and Skuterud 1982)
Amitriptyline	Equilibrium dialysis at 37°C for 6 hr	42	7.8 ± 1.0 (1.5-2-fold)	Spiked plasma from healthy volunteers. Similar results with nortriptyline (Brinkschulte et al. 1982)
Demethyl-chlorimipramine	CSF/plasma ratio	18	2.6 ± 0.7 (SD) (1.1-4.0)	15 depressed patients, 3 with obsessive-compulsive disorder, treated with chlorimipramine (Bertilsson et al. 1979)

Source: Sjöqvist et al. (1980a).

individual variability in the binding of nortriptyline and amitriptyline, a high correlation between individual binding of the two drugs, and a high correlation ($r = 0.99$) between unbound and total drug concentration in plasma.

Methodological difficulties in binding studies are particularly important to recognize for basic amines—e.g., the "Vacutainer artifact." Base binding in blood drawn in Vacutainer tubes is markedly and irreproducibly decreased owing to the release of substances from the stoppers that displace the drugs from α_1-acid glycoprotein (Borgå, Piafsky, and Nielsen 1977). Few studies have reported details regarding the procedures for collecting blood specimen.

Bertilsson et al. (1979) showed that equilibrium dialysis and ultrafiltration were unsuitable techniques for determining the binding of demethylchlorimipramine (DMCI) which also binds to lipoproteins in view of its lipophilicity. Its binding decreased substantially upon sustained equilibrium dialysis of plasma, presumably owing to destruction of some binding protein, This may occur owing to bacterial growth or to denaturation of certain proteins (e.g., lipoproteins) in plasma kept at 37°C for several hours.

It seems justified to conclude that interindividual differences in binding of TCA and some other basic drugs to plasma proteins are small compared with those demonstrated in steady-state plasma concentrations at fixed doses. Occasional patients may nevertheless have binding outside the commonly reported two-fold range due to concomitant diseases that change the concentrations of binding proteins. This has obvious implications for the interpretation of total drug levels in such patients. However, it will probably be difficult to demonstrate that unbound concentrations of TCA correlate better to clinical outcome than total concentrations in the light of the many pitfalls involved in evaluating antidepressant drug action. Hitherto, one such study has been published but the results were equivocal (Breyer-Pfaff et al. 1982).

SPECIAL PROBLEMS

Interindividual variability in drug binding may become a significant clinical problem, if drug binding is concentration-dependent. The old drug salicylic acid is such an example (Ekstrand, Alván, and Borgå 1979), where therapeutic drug monitoring probably should benefit from measurements of free concentrations. Other contributions to this book concern the concentration-dependent binding of disopyramide.

GENETIC FACTS

The possibility of genetically determined aberrations in drug binding should always be considered. For nortriptyline, a genetic component in binding has been demonstrated in twin studies (Alexanderson and Borgå 1972), but the role of environmental factors was also clear. The authors pointed out that all the twins lived in different households, which might explain the significant intrapair differences in binding in monozygotic pairs.

Alván et al. (1983) recently reported that the unbound fraction of propranolol varied from 1.9 percent to 13.2 percent in 434 plasma samples from members of 132 families (Fig. 11-3). The concentration of α_1-acid glycoprotein was an important determinant for the free fraction shown as the significant correlation between propranolol binding ratio and the glycoprotein concentration (Fig. 11-4). By applying path-analysis, 21 percent of the variability in propranolol binding could be ascribed to genetic factors and only 5 percent could be explained by environmental factors in common. Thus, the heritability of this trait was quite low and about three-quarters of the total variability in propranolol binding was unexplained. This includes methodological errors but also the fact that α_1-acid glycoprotein concentration is subject to random variability due to intercurrent infection.

The vast majority of the individuals in the study by Alván et al. (1983) had free propranolol fractions between 3 percent and 9 percent.

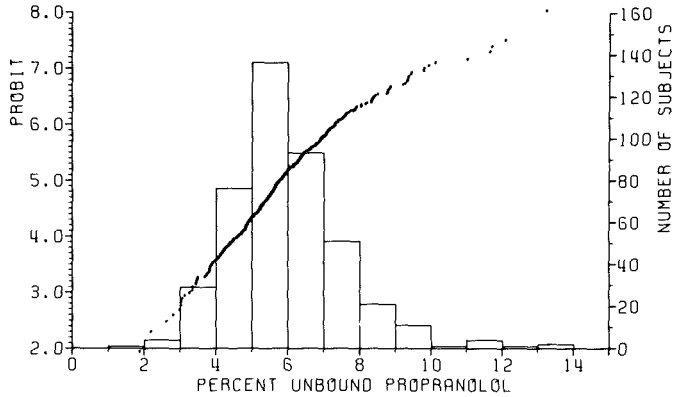

FIGURE 11-3
Distribution and probits of unbound propranolol in 432 plasma samples in a family study.
Source: Alván et al. (1983). Reprinted with permission.

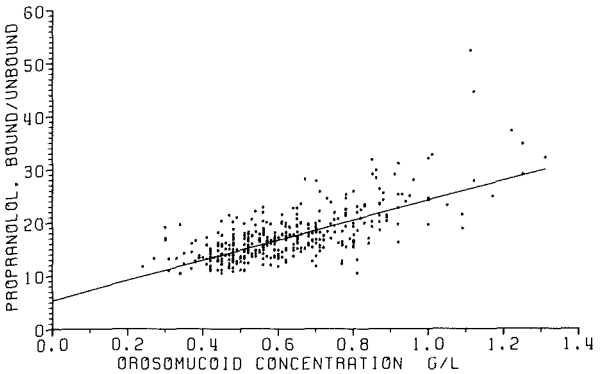

FIGURE 11-4
Correlation between the ratio of bound to unbound propranolol versus orosomucoid (α_1-acid glycoprotein) concentration in 406 plasma samples ($r = 0.67$, $p < 0.001$).
Source: Alvån et al. (1983). Reprinted with permission.

Qualitative differences in the structure of the binding proteins may explain the occurrence of occasional outliers in binding. In this particular study, reinvestigation of the few individuals exhibiting low binding (high free fraction), yet normal orosomucoid concentration, failed to show a consistent pattern (Alvån, personal communication).

SUMMARY

This literature survey suggests that interindividual variability in drug binding is small (usually two-fold) compared to that in other kinetic parameters such as steady-state plasma concentrations (often five- to ten-fold). This generalization is based on experience with the acidic drug phenytoin and basic drugs such as tricyclic antidepressants and probably holds for metabolically healthy individuals (normal kidney and liver function), who are not simultaneously treated with other drugs with displacement effect on the binding proteins. Exceptions to this rule are patients with pathophysiologically induced changes of plasma protein and drugs with concentration dependent binding within the therapeutic range. Therapeutic monitoring of unbound drug concentrations should always be based on results obtained in controlled clinical investigations.

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