Dose-response relationships in clinical pharmacology
L Lasagna, S Erill, C A Naranjo, editors

PHARMACOKINETIC MODULATION OF THE DOSE-RESPONSE RELATIONSHIP

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INTRODUCTION

The relationship between the administered dose and observed pharmacologic effects can be modified using a variety of techniques. For example, comparatively simple maneuvers such as slowing and prolonging oral or intramuscular absorption will decrease peak effects and increase the duration of action for some drugs. Such modulation of the delivery of the dose will yield an improved therapeutic outcome in a subset of patients for a number of compounds such as meperidine[1,5]. However, the principal purpose of this review is to summarize recent advances in our understanding of one particular form of modulation of the plasma concentration-effect relationship; that is, alteration in the free fraction of drug in plasma. The discovery that the plasma concentration of α_1-acid glycoprotein (AGP) is the major determinant of the free concentration of many important lipophilic basic drugs[2,3] allows us to understand the underlying mechanisms for a number of disease induced changes in pharmacokinetic behavior and concentration-effect relationships. Furthermore, the intravenous administration of proteins which have a very high affinity for digoxin has been shown to be a very valuable treatment to reverse the toxicity of this drug[4]. Thus, the coming together of these various factors which provide insight into the determinants of free concentration in plasma and observed pharmacologic effect provide the basis for this discussion.

OVERVIEW

The traditional theory describing the relationship between pharmacologic effect and free drug assumes that the free drug concentration in plasma (Cfp) is equal to the free drug concentration in tissues (Cft) containing receptor sites responsible for drug action. Thus, the drug in the body that is bound to blood constituents is believed to make no direct

*This research was supported in part by Grant GM 20852 from the National Institute of General Medical Sciences, National Institutes of Health and by American Foundation for Pharmaceutical Education Fellowships to JGB and JAB.

contribution to effect. This relationship between bound drug, free drug and pharmacologic effect is summarized in FIGURE 1. According to this generally accepted scheme, only unbound drug is capable of crossing biological membranes. Furthermore, for most drugs, only unbound compound is capable of interacting with receptors. Therefore, it can be assumed that plasma free drug concentration reflects the availability of drug for interaction at receptor sites in rapid equilibrium with plasma. Similarly, if steady state conditions exist, free drug in plasma should have the same relationship with receptor sites which equilibrate more slowly with plasma.

It must be noted, however, that this theory has been tested only in recent years, with only a modest number of studies directly examining its validity.

Studies in Patients and Normal Volunteers

In clinical practice, several studies have shown Cfp correlates well with the pharmacologic effect of certain antiarrhythmic[4] and antiepileptic agents[11]. However, only a few of these studies have shown that Cfp is clearly superior to total plasma concentrations (Ctp) as a predictor of drug effect or toxicity[7-9]. None of these studies can be said to have conclusively proven the validity of traditional pharmacologic effect theory. Thus, it is not surprising that the use of Cfp measurements in clinical practice is infrequent. Perhaps the most compelling data suggesting the value

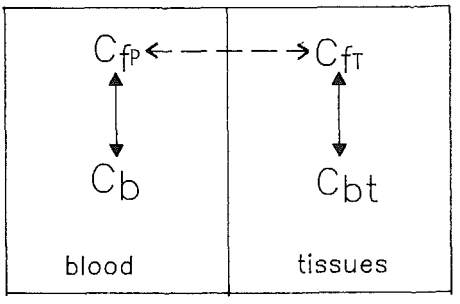

Fig. 1. The traditional assumed relationship between unbound (C_f) and bound (C_b) drug concentrations in plasma (blood) and tissues.

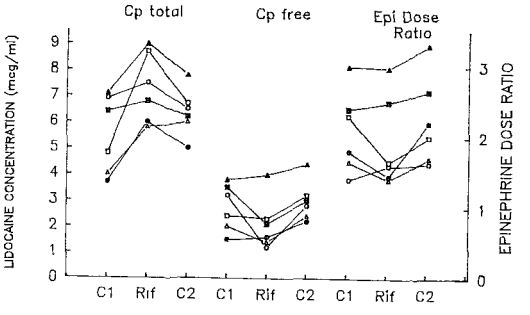

Fig. 2. Total and free plasma concentrations and epinephrine (Epi) dose ratio (after/before) after lidocaine treatment in six dogs on three occasions: before rifampin (C1), on the 14th day of rifampin (Rif): and 4 weeks after stopping rifampin (C2). Adapted from Ref 12 with permission of the Authors and Am Soc for Pharm Exp Ther.

of Cfp measurements is that of Oellerich in his studies of phenytoin toxicity[7]. He found that none of the patients he examined exhibited signs of toxicity when free phenytoin concentration was less than 2.3 mg/L and that all the subjects in his population without signs of toxicity had a free phenytoin concentration less than 2.7 mg/L. In contrast, there was a high degree of overlap in the toxic and non-toxic populations when they were segregated based on Ctp. As impressive as these findings are, it is probably appropriate to have them confirmed before endorsing the routine monitoring of free phenytoin concentrations, unless there is an obvious reason (renal failure, hypoalbuminemia, etc.) to suspect an abnormal free fraction.

EXPERIMENTAL APPROACHES FOR ALTERING DRUG PROTEIN BINDING AND PHARMACOLOGIC EFFECT

Mechanistic investigations of the relationship between Cfp and drug effect in laboratory animals have been reported for propranolol, lidocaine, disopyramide and several other drugs[12-14]. These investigators have utilized a number of approaches to modulate the ratio of free and total drug concentration in plasma and the results of these studies can be grouped by the technique used to modulate binding.

Use of Hepatic Enzyme Inducing Agents to Increase Binding Protein Concentration in Plasma

A number of drugs including rifampin and phenobarbital, which induce the synthesis of selected cytochrome P450 isozymes have been

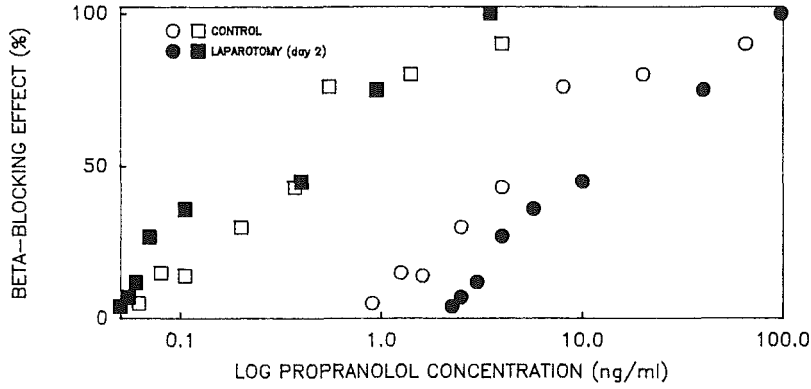

Fig. 3. Relationship between mean plasma concentration and β-blocking effect of propranolol (0.5 mg/kg IV) in control and laparotomized rats. Adapted from Ref 13 with the permission of the Authors and Am Soc for Pharm Exp Ther. Circles and squares represent total and free concentrations, respectively.

shown to increase the serum concentration of AGP in a species dependent manner (this is discussed in detail in a subsequent section of this text). This property has been used to study the effect of altered protein binding upon the pharmacologic effect of drugs.

For example, DeRick and colleagues have examined the effects of rifampin treatment upon the antiarrhythmic effect of lidocaine in dogs[12]. Each dog was studied before rifampin treatment; after 14 days of rifampin treatment (~20 mg/kg/day orally); and 28 days after completing this treatment. Arrhythmias were induced in anesthetized animals with intravenous epinephrine, using the observation of two or more ectopic beats in a 10 second period as the criteria for arrhythmia induction. The effectiveness of free and total lidocaine concentrations was reported as the ratio of the epinephrine dose needed to induce arrhythmias in the presence and absence of lidocaine.

The data in FIGURE 2 clearly indicate that lidocaine effect was not correlated with Ctp and that it was correlated with Cfp. However, the data in FIGURE 2 also suggest the response of individual animals is rather variable and a significant portion of this variability cannot be explained by differences in protein binding.

Similarly, Bai and Abramson have studied the influence of phenobarbital treatment upon the pharmacologic effect of single doses of propranolol in dogs[15]. Their findings clearly suggest that a reduction in propranolol Cfp was responsible, at least in part, for the decreased effect observed in phenobarbital pretreated animals.

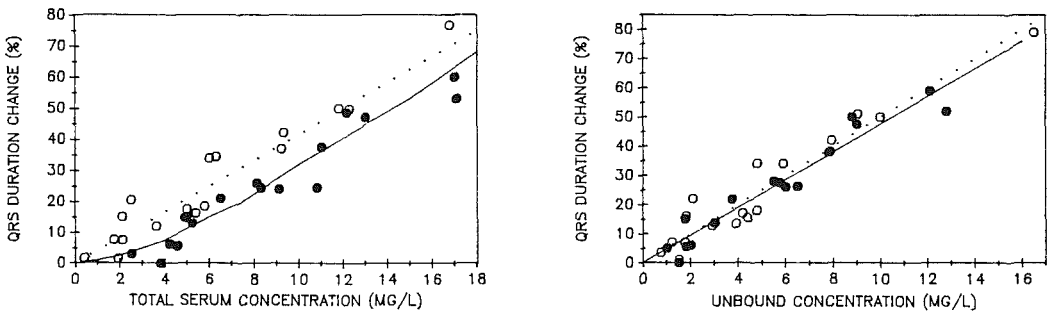

Fig. 4. Relationship between disopyramide serum concentration at steady state and the relative change in QRS duration in a representative rabbit. Adapted from Ref 14 with the permission of the Authors and Am Soc for Pharm Exp Ther. (•)AGP;(○)control

Thus, the use of enzyme-inducing agents to alter protein binding of basic drugs has been successfully employed to study the significance of Cfp and Ctp[12,15]. As described above, evidence has been presented which suggests Cfp is superior to Ctp as a predictor of response. However, such studies are complicated by the multiple effects of enzyme-inducers upon hepatic function, drug disposition (serum concentration of pharmacologically active metabolites) and any inherent pharmacologic effects of the inducer.

Use of the Acute Phase Response to Alter Protein Binding and Modulate Pharmacologic Effect

There have been several reports of the effects of inflammation and trauma on drug disposition[13,16-19]. These changes are thought to occur secondary to the acute phase response[19] (discussed in detail in a subsequent section). Briefly, following trauma or inflammation, the effect of zoxazolamine, hexobarbital, and propranolol have been shown to be altered secondary to pharmacokinetic changes[13,17]. Furthermore, Belpaire et al[16] and Yasuhara and associates[13] have recently attempted to directly investigate the influence of the acute phase response upon protein binding, Cfp and the relationship of these parameters to pharmacologic effect.

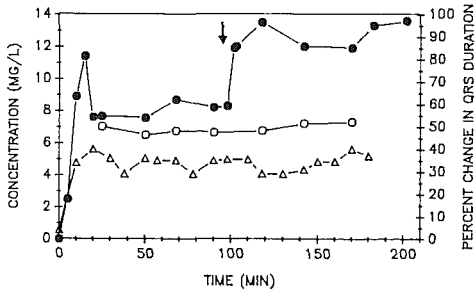

Fig. 5. Relationship of R-disopyramide total (●) and unbound (○) serum concentrations with the change in QRS duration (△) in a rabbit. Partially purified human AGP was injected (40 mg/kg) at the indicated time (▼). Adapted from Ref 20 with the permission of the Authors and PJD Publications, Ltd, Westbury, NY, USA. Copyright c by PJD Publications, Ltd.

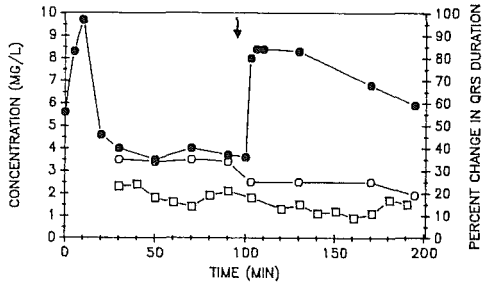

Fig. 6. Relationship of S-disopyramide total (●) and unbound (○) serum concentrations with the change in QRS duration (△) in a rabbit. Partially purified human AGP was injected (80 mg/kg) at the indicated time (▼). Adapted from Ref 20 with the permission of the Authors and PJD Publications, Ltd, Westbury, NY, USA. Copyright c by PJD Publications, Ltd.

Belpaire et al examined the ability of beta-adrenergic blockers to inhibit isoproterenol induced increases of heart rate in pithed rats[16]. They studied propranolol and oxprenolol (AGP binders), as well as atenolol and metoprolol (which do not bind significantly to AGP), using turpentine injections to induce inflammation. After intravenous administration, propranolol and oxprenolol had significantly less beta-blocking effect in rats with inflammation when compared to control rats. Furthermore, there was no difference in the beta-blocking effect of metoprolol and atenolol when comparing control rats to those with inflammation. As expected, propranolol treated rats with inflammation had significantly elevated Ctp and decreased Cfp values (due to increased protein binding). A significant positive correlation was found between log Cfp of propranolol and the intensity of beta blockade (r=0.687).

Yasuhara et al have demonstrated the effects of surgical laparotomy upon propranolol disposition in rats[13]. They examined the effects of laparotomy on the ability of propranolol to inhibit isoproterenol induced elevations of heart rate. They observed that the effect versus log Ctp curve was displaced to the right in laparotomized rats as compared to control animals (FIGURE 3). Superficially, this suggests that laparotomy is associated with a

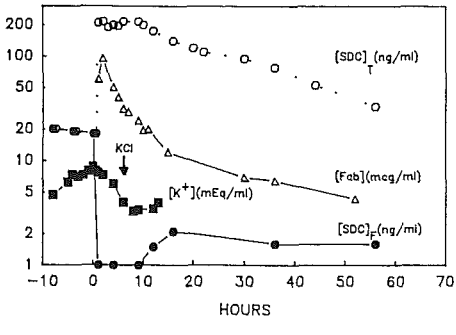

Fig. 7. The concentration time course of serum potassium , total serum digoxin ([SDC]T;), free serum digoxin ([SDC]F;), and sheep digoxin Fab fragments ([Fab];). KCl indicates oral doses of KCl (120 mEq over 6 hours). Adapted from Ref 2 with the permission of the Authors and the New England Journal of Medicine.

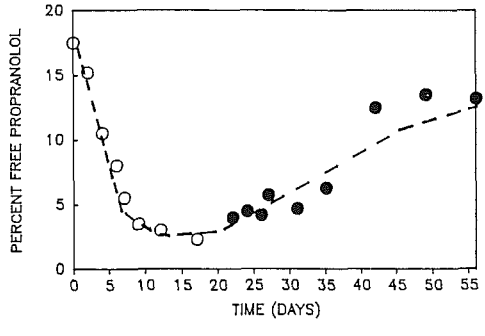

Fig. 8. Propranolol protein binding in dogs treated with phenobarbital (180 mg/day p.o.) on days 0 through 20. Data are the average (Std error bars omitted) from single measurements in each of four dogs. Adapted from Ref 6 with the permission of the Authors and Am Soc for Pharm Exp Ther.

decreased sensitivity of the myocardium to propranolol. However, the effect versus log Cfp curves were virtually identical in control and laparotomized animals. Thus, an elevated AGP concentration resulting in increased bound propranolol concentration appears to account for the shift in the effect versus log Ctp relationship. This study clearly illustrates the need to account for altered protein binding when studying the pharmacologic effect of basic drugs in disease states as well as in animal models involving surgical interventions.

Alteration of Drug Binding and Effect Using Protein Infusions

The infusion of AGP to enhance the plasma protein binding of basic drugs has been described by Huang and Øie[14,20]. One important aspect is that several investigators have found little or no physiologic or toxic effects of human AGP infusions in animals[14,20-22]. Thus, this approach appears to be desirable for the study of the influence of Cfp upon drug effect as it avoids the many physiologic alterations associated with enzyme-inducing agents and inflammation.

Huang and Øie have investigated the pharmacologic effect of disopyramide (QRS duration) as a function of Cfp and Ctp[14]. Infusions of partially purified human AGP were used to alter the ff of disopyramide in rabbits. It was found that effect was significantly correlated with Ctp in both control and AGP infused animals when data from these two groups were analyzed separately (FIGURE 4). However, the effect versus Ctp relationships of control and AGP infused animals were significantly different, with much more effect observed for a given total plasma concentration in the control animals. Effect also correlated significantly with Cfp in both control and AGP infused animals (FIGURE 4)[14]. Significantly, there was no difference between the relationship of effect and Cfp between the AGP treated and control groups. Since the shift of the effect versus Ctp curve was similar in magnitude to the increase in Ctp secondary to elevated AGP, the authors concluded that disopyramide pharmacologic effect was determined by Cfp. In a second experiment, Huang and Øie studied the influence of the same partially purified AGP preparation on ff of disopyramide using each rabbit as its own control[20]. The results of this study were in agreement with their first report (see FIGURES 5 and 6).

Recently, Chiang and Øie reported prazocin induced blood pressure reduction as a function of Cfp[21]. Using bolus or infusion doses, they found 35% greater unbound prazocin concentrations were required to elicit similar blood pressure reductions in AGP infused rats when compared to control rats. The results of the prazocin study are not yet explained, as AGP infused alone had no effect upon blood pressure. However, even these observations highlight the utility of this model for investigating the influence of protein binding upon the pharmacologic response of drugs.

Use of Drug Specific Antibodies and Fab Fragments to Alter Plasma Drug Binding and Pharmacologic Effect

The use of specific antibodies and/or Fab fragments for the treatment of drug intoxication has received growing attention over the last decade. Digoxin specific Fab fragments have proven to be a highly effective therapy in the management of digoxin overdose in humans[23,24]. More recent work has been aimed at developing similar therapies for tricyclic antidepressants and phencyclidine[25,26].

The reported experiences with digoxin specific Fab fragments can provide insight into the importance of Cfp in determining and predicting pharmacologic effect. Smith et al reported the first clinical use of Fab fragments for treatment of digoxin toxicity[2]. The time course of total and free serum digoxin concentrations and Fab fragment concentration is described in FIGURE 7. Starting at time 0, a 2 hour infusion of a dose of Fab fragments approximating the number of moles of ingested digoxin was begun. The serum digoxin concentration at time 0 was estimated to be 20 ng/ml and all of this was free drug. Within 1 hour of starting Fab fragment therapy, total digoxin concentrations rose to 200 ng/ml (10 fold increase), while the unbound concentration was not detectable (less than 0.5 ng/ml). At the same time, positive clinical and EKG responses were noted. Improvement continued throughout the infusion and the patient appeared out of danger from life threatening arrhythmias by 10 minutes after completing the Fab infusion. Many case reports have since beenpresented with similar findings[23,24].

However, Fab fragment results may not be directly extrapolated to the case of simple drug-plasma protein binding since the association of drugs with Fab fragments has different characteristics than typical drug-plasma protein interactions. For example, affinity constants (Ka) on the order of $10^9 M^{-1}$ have been

reported for the binding of drugs to specific Fab fragments[26] while Ka values for drug binding to plasma proteins are typically about 10^5 M^{-1}[27]. Thus, binding to Fab fragments may be much more slowly "reversible" than classical drug-plasma protein binding.

In closing this section, it should be noted that Pardridge and coworkers[28] have provided significant evidence suggesting that transport through some biological membranes of certain plasma protein bound ligands (hormones and drugs) is much more rapid than would be expected based on the conventional assumption that the concentration of drug-protein complex does not contribute to the instantaneous driving force for moving drug across such barriers. The significance of this observation in the context of drug effect remains to be established. However, it is clear that its potential importance could be enormous.

RECENT ADVANCES IN OUR UNDERSTANDING OF THE FACTORS WHICH CONTROL α_1-ACID GLYCOPROTEIN PLASMA CONCENTRATION

Increases in the serum concentration of AGP and the associated decrease in the ff of many basic lipophilic drugs often accompany such events as tissue infarction, burns, inflammation and trauma[3,29-31]. However, it has only recently been reported that the administration of certain drugs known to be inducers of various hepatic cytochrome P-450 isozymes results in a similar response (but only in selected species and indeed with significant inter-strain and gender differences). The increases in AGP concentration following the administration of enzyme inducing drugs may be of clinical importance since these drugs (phenobarbital, phenytoin, rifampin) and basic lipophilic drugs such as propranolol, disopyramide and lidocaine (which bind significantly to AGP) are often coadministered to patients. Decreases in the Cfp of basic lipophilic drugs resulting from increased binding to AGP could result in inadequate effect (see above) as well as altered drug disposition. Further, from the limited number of studies reported to date, it appears that the response of AGP concentration to the administration of enzyme inducing drugs is species specific. It is imperative that more studies be performed in order to obtain a better understanding of the mechanisms involved in this phenomenon. It has been reported that after five days of treatment with phenobarbital (50 mg/kg, twice daily, po) the ff of desipramine fell to 59% and 73% of control values in male Wistar and Sprague-

Dawley rats, respectively[32]. The response to phenobarbital pretreatment was less pronounced in female Wistar rats (81% of control) and a statistically significant response could not be documented in female Sprague-Dawley rats[32]. This data suggests that the effect of phenobarbital on protein binding is both strain and gender dependent.

Similar observations have been reported by Chauvelat-Maochon et al who measured AGP serum concentration and the binding ratio (BR; the ratio of bound and free concentration) of propranolol after seven days of phenobarbital (70 mg/kg/day, sc) administration to male Sprague-Dawley rats as well as male and female Dark Agouti (DA) rats. The male Sprague-Dawley rats exhibited a 1.6 fold increase in serum AGP concentration and a 1.3 fold increase in propranolol BR. A more marked response was observed in DA rats with males having a 6.9-fold increase in serum AGP concentration and a 3.1-fold increase in propranolol BR. Female DA rats had a 4.4 and 1.9 fold increase in AGP concentration and BR, respectively[33]. In a related study, other investigators have reported that the serum AGP concentration was significantly higher in rats receiving phenobarbital (1 g/l in drinking water for 6 days) than in controls (0.62 ± 0.08 vs 0.32 ± 0.03 mg/ml)[34]. This increase in AGP was accompanied by a significant decrease in propranolol ff.

The effects of enzyme inducing drugs on serum AGP concentration and the protein binding of basic lipophilic drugs in humans is less clear and is currently a topic of active investigation[35-39]. One group of investigators has reported that serum AGP concentration was similar in normal healthy volunteers and adult epileptic patients receiving long term therapy with either phenobarbital or carbamazepine[35]. Indeed, they observed that patients receiving phenobarbital and carbamazepine exhibited a significant decrease in serum AGP concentration. In contrast, Tuila and Neuvonen[36] reported increases in AGP concentration (58% and 23% higher than controls, respectively) in adult epileptic subjects receiving phenytoin or carbamazepine. Increases (36% greater than control) in AGP concentration have also been observed in epileptic children receiving carbamazepine alone[37]. The serum protein binding of amitriptyline (known to bind to AGP) was not significantly altered in adult patients with seizure disorders receiving combinations of phenobarbital and other anticonvulsants[32]. In normal healthy

smoking and nonsmoking volunteers, Kapil et al[38] noted that typical enzyme inducing doses of phenobarbital (100 mg/d po for 21 days) did not effect serum AGP concentration or disopyramide protein binding. Thus it appears that man does not reliably respond to anticonvulsants in a manner similar to that observed in rats though some evidence suggests selected anticonvulsants influence serum AGP level.

The administration of phenytoin or barbiturates to the dog has been reported to reliably increase the serum protein binding of propranolol and lidocaine[6,40]. After a treatment period of at least three weeks with phenobarbital (180 mg/d po), Bai and Abramson[6] observed a dramatic fall in the ff of propranolol (0.152 ± 0.012 vs 0.024 ± 0.003; FIGURE 8). Mean peak and trough phenobarbital plasma concentrations were determined and were found to be similar to those seen in human epileptics. When other dogs received three weeks of treatment with phenytoin (200 mg po twice daily), the ff of propranolol fell to an extent similar to that observed following phenobarbital administration. Indeed, when Edwards et al[40] studied the effect of a single anesthetic dose of pentobarbital (26 mg/kg iv) on the protein binding of lidocaine in the dog, they noted a significant decrease in the ff of lidocaine two days after pentobarbital administration with a slow return to

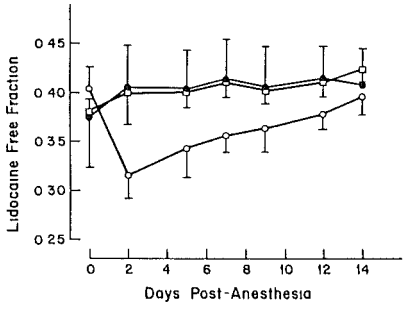

Fig. 9. Mean lidocaine free fraction (± 1 SD) in dogs following sodium pentobarbital (o) 26 mg/kg (n=8), chloralose (□) 100 mg/kg (n=3), or no treatment (●) (n=4). Reproduced from Ref 40 with permission of the Authors and the copyright owner, the American Pharmaceutical Association.

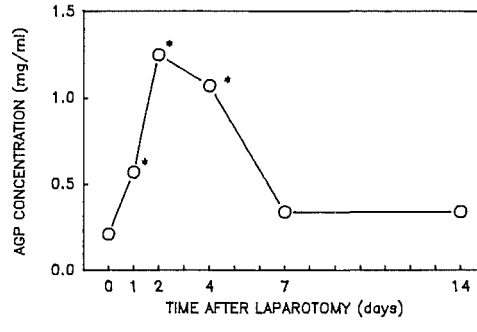

Fig. 10. Plasma-concentration time course of rat AGP after laparotomy. Each point represents the mean (Std error bars omitted) for more than five animals. (P<.001 vs. control). Adapted from Ref 13 with the permission of the Authors and Am Soc for Pharm and Exp Ther.

pre-dose free fractions 14 days later (FIGURE 9). Interestingly, the administration of the nonbarbiturate anesthetic chloralose (100 mg/kg i.v.) had no effect on the ff of lidocaine[40].

The antibacterial drug rifampin is also a potent inducer of cytochrome P-450 in man[41,42], dogs[43] and mice[44]. However, rats[44] and guinea pigs[45] are far less responsive to such treatments. The effects of rifampin treatment on AGP concentration and protein binding has been examined in man and dog and has been shown to differ significantly. Both species exhibit profound induction of oxidative metabolism but only the dog demonstrated the characteristic fall in ff associated with an increase in AGP. Specifically, Feely et al[39] examined the effect of rifampin (600 mg/d x 14-21 days) administration on AGP concentration and lidocaine ff in normal volunteers and found no effect. These investigators also examined the effect of chronic rifampin treatment (600 mg/d with isoniazid 300 mg/d for a mean 5 months) and again found no effect on lidocaine binding or serum AGP concentration[39]. Herman et al[46] reported similar findings at about the same time. In contrast, Belpaire et al[43] observed a large decrease in both propranolol ff and oxprenolol ff following rifampin administration to dogs. Furthermore, Delcroix and coworkers[47] reported a significant negative correlation between AGP concentration and the free fraction of both propranolol and oxprenolol in dogs receiving rifampin (600 mg/kg po twice daily x 12 days). Thus, the available evidence suggests that the signal for the increased synthesis of AGP and the enzymes of the cytochrome P-450 system may be under some degree of shared control in the dog but perhaps not in man.

Since anesthesia with barbiturates is often used when performing surgery on larger laboratory animals, it is appropriate to note that the protein binding of basic lipophilic drugs is also profoundly affected by surgical and other experimental procedures. Furthermore, it is essential for experimental pharmacologists to understand the temporal sequences of these changes in plasma protein binding. For example, Terao and Shen[48] examined the effect of the presence of an indwelling jugular vein catheter on the protein binding of propranolol in the rat. The propranolol serum ff was not significantly different in sham operated control rats 1 or 7 days after surgery. In contrast, rats with indwelling catheters had a 43% decrease in propranolol ff between day 1 to 7

after surgery and there was no further decrease in propranolol serum ff from day 7 to 14 after surgery. Furthermore, in rats whose catheters were removed 7 days post surgery, the propranolol serum ff returned to near control values by day 14. These data provide significant insight into the time course of changes in AGP concentration associated with such experimental interventions.

In more recent studies, Yasuhara et al[13] examined the effect of laparotomy on AGP concentration in the rat and observed a marked rise in plasma concentration with a maximal response occurring about 2 to 4 days after surgery (FIGURE 10). By day 7 after surgery, AGP concentrations had returned to control levels. In another study, surgical procedures involving implantation of cannulas into the femoral vein and artery as well as the urinary bladder and bile duct were performed on control and phenobarbital-treated rats (1 g/l in drinking water for 6 days)[34]. Over a 60 hour period, the time course of change in AGP concentration and propranolol protein binding following surgery was similar in both groups with increases becoming evident approximately 11 hours following surgery and a plateau occurring by about 30 hours post intervention.

From a different perspective, the inflammatory response induced by turpentine has been shown to result in a dramatic elevation of serum AGP concentration. In rats given a single dose of turpentine the serum concentration of AGP increases about fivefold with a maximal increase occurring between 48 to 72 hours[49] post dose. When compared to controls, Belpaire et al[16] observed a significant decrease in the propranolol ff in rats which had received turpentine (0.5 ml, im) 24 and 48 hours earlier (0.139 ± 0.012 and 0.024 ± 0.003, control vs treatment). Similarly, 48 hours after the subcutaneous administration of turpentine (0.5, 1.0 and 3.0 ml/kg), quinidine ff decreased to 43%, 37% and 22% of control, respectively, in male Sprague-Dawley rats[24]. This decrease in quinidine ff was accompanied by a significant increase in the serum concentration of sialic acid (a component of AGP) which is indicative of an increase in the serum concentration of AGP[50].

SUMMARY

The absolute proof that Cfp is significantly better correlated with drug effect than is Ctp is generally not available from clinical studies. However, from such studies significant

suggestive data does exist. From experiments performed in laboratory animals, much more substantial evidence has been gathered. New techniques (i.e. Fab fragments) and an understanding of the factors which control the plasma concentration of acute phase response proteins (eg. biologic response modifiers) should lead to significant advances in this field in the next decade.

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Discussion - Pharmacokintic regulation of the dose-response

E. Perucca

I was rather puzzled by the differences in the response of $alpha_1$ acid glycoprotein to enzyme inducers in man elicited by different studies. I wonder whether there is any evidence that genetic variation contributes to response to inducers. On the other hand, one should perhaps study possible circadian variations in $alpha_1$ acid glycoprotein levels, which might blur in some studies the effect of the inducers.

D. Lalka

There is one report showing substantial intra-day variability in $alpha_1$-acid-glycoprotein levels in individual normal subjects, but I am unaware of anyone who has tried to repeat that study. In any event, let me mention that we have studied the increase of $alpha_1$-acid-glycoprotein levels in the first 5 to 7 days following major trauma. In about 15 subjects and we have observed that the response is fairly predictable and that it does not seem to be under particularly strong genetic control.

A. Reinberg

I just would like to mention that circadian rhythms in the baseline values of $alpha_1$ acid glycoprotein and on the effects of inducers on drug metablism have been very well documented. I am sure that a study of the effects of inducers on AAG levels in rats would show quite different results in animals treated during the day or the night.

B.P. du Souich

In any event, one should remember that some inducers, such as phenobarbital, have very long elimination half-lives, and sustained presence of high plasma levels would shut-off any circadian effect.

L. Lasagna

We all know that pharmacokinetic changes are not necessarily accompanied by pharmacodynamic changes. From the practical standpoint, what we are concerned about is whether something will

change the relationship between dose and response. It is a little frustrating to have all these data that so nicely demonstrate changes in kinetics without really much in the way of accompanying pharmacodynamic data. I just wondered whether you had any data up your sleeve that you weren't showing us that would convience some of us that some of these changes are going to be practically important.

D. Lalka

In terms of clinical data there is very little, except the impressive results of the use of Fab fragments in cases of severe digoxin toxicity. As far as animal data are concerned, results are available with regard to propranolol free fraction and degree of beta blockade, as well as for disopyramide and arrhythmia supression.

R.J. Temple

Perhaps it is worth mentioning that some of the early surprisingly devastating responses to disopyramide were thought to be at least possibly related to the ability to get a large increase in free disopyramide with only modest increases in daily dose.

D. Lalka

Among therapeutic agents, disopyramide is the one which exhibits the greatest variation in free fraction as the total concentration is increased in the clinical concentration range. There are a few other compounds that show modest changes, but nothing nearly as dramatic as disopyramide.