PHARMACOKINETICS OF PD123,497, A NOVEL ANTIARRHYTHMIC DRUG: 
EARLY DISTRIBUTION PHASE IN BLOOD AND TISSUES 

by 

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PD123,497 is a novel antiarrhythmic drug and the (+) enantiomer of PD117,302, a kappa (κ) opioid agonist. Using a method of liquid-liquid chemical extraction and high performance liquid chromatography, the pharmacokinetics of PD123,497 in the rat were determined. Specifically, concentration-vs-time profiles were constructed reflecting the early distribution phase of an intravenous bolus dose of 8 μmol/kg in whole blood, heart, liver, brain, and skeletal muscle. The pharmacokinetic data for heart and blood correlated well with the time course of heart rate and blood pressure effects as determined in previous pharmacological assays. The data also correlated well with pharmacological screens in dogs and baboons indicating that the rat model is demonstrative of the situation in other species. Skeletal muscle data indicated a significant capacity for drug accumulation in the muscle beds. Liver data indicated slow uptake and storage of parent drug. Termination of therapeutic effect appeared to be the result of redistribution into other tissues and not metabolism or elimination. Concentrations in the heart were several times higher than that of the blood immediately after administration of drug via the tail vein. This suggests a potential for alpha-phase toxicity. Brain data showed that peak concentration was achieved after that in the heart indicating redistribution from the heart and suggesting that PD123,497 may be able to produce significant CNS side effects.
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LIST OF ABBREVIATIONS

PVB.................. premature ventricular beat
MI.................... myocardial infarction
AA.................... antiarrhythmic drug
\( V_{\text{max}} \)........... maximum rate of depolarization
. (phase 0 of the cardiac action potential)
ERP................... effective refractory period
CNS................... central nervous system
CAST.................. cardiac arrhythmia suppression trial
RSD................... Rhythm Search Developments Ltd.
\( K_a \).............. apparent constant of association
\( V_d \)............... apparent volume of distribution
\( K \)............... elimination rate constant
\( T_{1/2} \)............ elimination half-life
HPLC.................. high performance liquid chromatography
MtBE.................. methyl-tertiary-butyl-ether
SEM.................. standard error of the mean
ACKNOWLEDGMENTS

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INTRODUCTION

This thesis was part of a programme related to the development of new antiarrhythmic agents (the "RSD compounds"). Structure-activity relationship (SAR) studies on the various RSD compounds have been underway for some time. Some of these compounds are structural analogues of PD117,302, a selective κ-opioid agonist with antiarrhythmic activity. This thesis is concerned with the pharmacokinetics of PD123,497, the (+) enantiomer of PD117,302. We specifically investigated the early phase of distribution of PD123,497 in blood and tissue and related this to previous pharmacological studies done with this drug. This introduction provides the reader with information on Class I antiarrhythmics, the importance of pharmacokinetic information in the design and presentation of a drug for clinical use, the analytical methods used to obtain some of this information, and where our research is likely to lead.

I. THE CLASS I ANTIARRHYTHMICS:

a) Background information:

Sudden cardiac death is a leading cause of death in North America. Over 80% of these deaths are due to ventricular fibrillation. This fatal type of arrhythmia may be preceded by premature ventricular beats (PVB's). Patients who have suffered a previous myocardial infarction (MI) are at a high risk of fatal arrhythmia and so are often prescribed antiarrhythmic drugs (AA's).

It was assumed that there was a strong association between PVB's and
subsequent mortality and so PVB’s were considered to be the prime target of antiarrhythmic therapy. In consequence, suppression of PVB’s was considered the defining factor of drug efficacy. Studies conducted so far do not indicate that suppression of PVB’s results in decreased mortality for post-MI patients [1,2]. In fact, the Cardiac Arrhythmia Suppression Trial (CAST) of 1989 [3] reported an increased incidence in mortality for post-MI patients taking encainide and flecainide, a 3.6-fold difference compared to matched post-MI patients receiving placebos. It is now a well-established fact that the Class I drugs have significant pro-arrhythmic effects, although electrophysiologists predicted this many years ago [4,5,6]. The search for an effective antiarrhythmic drug continues.

b) Classification of antiarrhythmics:

The antiarrhythmic drugs are grouped according to their electrophysiological effects and presumed mechanism of action (see Table 1). This classification scheme was originally described by Vaughan Williams [7]. There are also subclassifications within groups based on their effect on $V_{\text{max}}$ (defined as the rate of change of voltage potential during phase 0 of the action potential).

**TABLE 1: Classification Scheme of Antiarrhythmic Drugs**

<table>
<thead>
<tr>
<th>CLASS</th>
<th>subclass</th>
<th>action</th>
<th>examples</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>a</td>
<td>moderate $\downarrow V_{\text{max}}$</td>
<td>quinidine</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>minimal $\downarrow V_{\text{max}}$</td>
<td>mexiletine</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>marked $\downarrow V_{\text{max}}$</td>
<td>encainide</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>beta-adrenoceptor blockade</td>
<td>propranolol</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>prolong repolarization (primarily potassium channel blockers)</td>
<td>amiodarone</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>calcium channel blockade</td>
<td>verapamil</td>
</tr>
</tbody>
</table>
c) Mechanism of action of Class I drugs:

An arrhythmia is an alteration in the normal sequence of activation of the atria and ventricles caused by a disturbance in conduction or an abnormality of rate, regularity, or site of origin of the cardiac impulse. The net effect of some antiarrhythmic drugs (notably the Class Ib drugs) is to prolong the effective refractory period (ERP) relative to action potential duration. This reduces the likelihood of generation of abnormal action potentials among the normal frequency of action potentials as set by the pacemaker.

Class I antiarrhythmics act on the sodium channel responsible for the initial inward current that constitutes the rising phase of the action potential. Much of our current functional understanding of the sodium channel is based on the work of Hodgkin and Huxley [8] who first demonstrated that a transient inward current of sodium ions was responsible for action potential propagation in squid giant axons. They characterized three states of the channel: open, inactivated, and closed.

Historically, the first drug with antiarrhythmic action discovered was quinidine and related compounds. At concentrations ten to one hundred times their antiarrhythmic concentrations these drugs are local anaesthetics in nerves. They were found to have the common property of "interfering specifically with the process by which depolarizing charge is transferred across the membrane" [9]. This action was revealed as a depression of $V_{\text{max}}$ unless the interstimulus interval was so long that it permitted full recovery between beats. It was suggested that these AA's interfered with the process by which sodium channels were "reactivated in response to repolarization" [9] and that consequently they "extended the effective refractory
period to a point long after the time at which repolarization was already complete" [9].
We now know these conclusions to be substantially correct.

The mechanism of action of the class I antiarrhythmics (and possibly other ion channel blocking antiarrhythmics) is currently best described by the modulated receptor hypothesis, postulated by Hille [10] and Hondeghem and Katzung [11] in 1977. At the time it was known that the decrease in $V_{max}$ due to quinidine was accentuated at high frequencies of stimulation and attenuated by slow rates and that this effect was observed as a stepwise decrease in $V_{max}$ with each action potential until a steady-state level of depression was achieved. The modulated receptor hypothesis accounts for these observations by postulating that the affinity of drug for the sodium channel is much greater when the channel is in an open or inactivated state than when it is in the resting state (closed). This necessitates cycling of the channel for drug action to occur and thus explains use- or frequency-dependence seen with some Class I drugs. The higher the rate of stimulation the more channels pass through open or inactivated states, have drug bound to them, and contribute to an overall decrease in sodium conductance. A steady-state level of $V_{max}$ depression is therefore reached sooner (in fewer beats). The modulated receptor hypothesis also states that the interaction between drug and channel receptor is voltage-dependent; that is, the drug has a higher affinity for the channel the higher the membrane potential. Thus when membrane potential is increased (depolarized) the drug is more readily bound and blockade of sodium current is more effective. Furthermore, a drug-associated channel inactivates more readily than drug-free channels and this also contributes to an effective decrease in sodium conductance. In cases of ischaemia,
the resting membrane potential is elevated, channel affinity for the drug is increased, and the time to recover from block is thereby increased resulting in a longer effective refractory period.

Another theory, the guarded receptor hypothesis [12] is a variation on the modulated receptor hypothesis and has not been ruled out at this point. Inherent in this theory is an explicit acceptance of the Hodgkin-Huxley model, the assumption that drugs bind in the sodium channel, and that drug-associated channels do not conduct sodium ions. Where it differs from the modulated receptor theory is a proposal that affinity of the drug for its binding site is constant (i.e. independent of channel state) and that in the closed state drug access is restricted. Thus there is more binding of drug in the open state than in the closed one. Drug binding is proposed to immobilize channel gates thereby blocking sodium influx. The frequency- and voltage-dependent nature of the gates would explain the frequency- and voltage-dependent nature of drug interaction with the sodium channel.

The use-dependent block of antiarrhythmics described above requires that the interval between stimuli be shorter than the time required for the channels to reactivate and recover from channel block. This allows for some specificity since in tissues following normal sinus rhythm there is enough time for recovery so that \( V_{\text{max}} \) should be only minimally affected, whereas PVB’s result in a shorter diastolic interval. The specificity for arrhythmogenic tissue is also enhanced by the usual relative depolarization of these tissues (as in the case of ischaemia) which increases the affinity of the channel binding site for the drug and slows channel recovery time.
d) Goals of our research (rationale):

From the results of the CAST study it is evident that current cardiac arrhythmia therapy with Class I antiarrhythmics is suboptimal and that it is necessary to develop channel blockers for at least three main reasons as put forward by Tamargo et al [13]. First, the population of people who need to be treated for supraventricular and ventricular arrhythmias averages almost three million patients in the United States [14]. Second, AA's are likely to remain the mainstay of therapy for the majority of patients with cardiac arrhythmias even when electrical (automated implantable defibrillator, anti-tachycardia pacemakers) and surgical strategies (ablation) can replace AA's in selected patients. Third, it is necessary to find better drugs than those currently being prescribed with less adverse side effects and higher therapeutic efficacy.

Many of the Class I antiarrhythmics in clinical use produce significant side effects. For example, quinidine not only blocks sodium channels but also muscarinic cholinergic receptors. Dysopyramide also has an anti-muscarinic action and may in addition block calcium channels. Procainamide can produce a syndrome that is similar to systemic lupus erythematosus. Class Ib agents such as lidocaine, phenytoin, tocainide, and mexiletine all have CNS side effects and the latter two drugs can cause clinically significant adverse gastrointestinal effects as well. Therapy with encainide and flecainide, both class Ic agents, was shown in the CAST study to increase the risk of sudden cardiac death and cardiac arrest. Another Class Ic drug, propafenone, can cause granulocytopenia.
The RSD antiarrhythmic project is concerned with developing new AA's with specificity for ischaemic cardiac tissue, a wide therapeutic margin, and minimal side effects. Compounds are synthesized using structural leads from a prototype molecule with unusual channel blocking actions and antiarrhythmic effects. As part of this project, pharmacokinetic determinations of blood and tissue concentration for one of the lead compounds provided information which will aid in analyses of in vivo screens by relating pharmacological effects to tissue and blood concentration. Information was also obtained with regards to maximum tissue concentrations (possible toxicity), rate of clearance, and time to reach steady state. These data are useful in considering drugs as clinical candidates. As quoted from Goodman and Gilman: "optimal therapy of cardiac arrhythmias requires an appreciation of the pharmacokinetic properties of antiarrhythmic drugs".

II. PHARMACOKINETICS:

The success or failure of drug therapy is largely dependent on dose regimen. The therapeutic goal is to achieve the optimum concentration of drug at the desired target which produces maximal therapeutic effect with minimal adverse effects. Knowledge of a drug's pharmacokinetic profile is essential to determine the relationship between concentration at the target tissue and administered dose, and to predict possible interactions with other drugs. Kinetic data also provides information about speed of onset and duration of effect. For many drugs a complete
pharmacokinetic profile is unknown or unavailable, and such information is obtained in the field, sometimes at the expense of the patient.

a) History of Pharmacokinetics:

Pharmacokinetics is defined as "being concerned with the study and characterization of the time course of drug absorption, distribution, metabolism, and excretion, and with the relationship of these processes to the intensity and time course of therapeutic and adverse effects of drugs." [15]. The term "pharmacokinetics" was first introduced by F.H. Dost in 1953 [16] although the subject matter had been discussed previously. A. Buchanen in England in 1847 [17] discussed absorption of ether from arterial blood to the brain, and speed of recovery as relating to redistribution to other parts of the body. Michaelis and Menten in Germany in 1913 [18] published the classic equation for describing enzyme kinetics, the concepts of which are also used to describe the elimination kinetics of ethanol, salicylate, and several other drugs.

From such beginnings pharmacokinetics grew into a distinct scientific discipline. With the development of improved analytical methods it has developed beyond the level of theory and inquiry into physiology and the body's handling of drugs to become a valuable tool in drug development and in the clinical setting to improve therapeutic uses of drugs [15].
b) The drug in circulation:

Absorption from the site of administration and transport into the blood is the first major step involved in the processing of drugs by the body. It is dependent on the drug's ability to cross various cell membranes. This is irrelevant of course in the case of intravascular administration. Many small molecule drugs can freely cross the vascular membrane, and since the entire volume of blood in the rat circulates within one minute, absorption into the blood from other sites of administration is usually rapid.

In blood the drug partitions between erythrocytes and plasma. While in the plasma fraction it can be protein-bound or free. Drug binding to red blood cells seldom has a pronounced effect on the body's handling of drugs, whereas binding to plasma protein does. Different drugs bind to different kinds of plasma protein. If the tested drugs are protein-bound in the plasma, they most likely interact with $\alpha_1$-acid-glycoprotein. Since the binding of many drugs to plasma protein depends upon hydrophobic interactions it may be regarded as a partitioning phenomenon between the aqueous phase of body water and the hydrophobic part of the plasma protein [19].

Binding to plasma macromolecules involves weak interactions; the energies of dissociation of such bonds differ considerably from those of covalent bonds which are typically about 150 kcal/mol as opposed to 1 - 10 kcal/mol for the former. Binding affinity is described by the apparent constant of association, $K_a$, which is the ratio of the rate constants $k_1$ and $k_2$ (for binding to and release from the protein, respectively).
The higher $K_a$, the greater the affinity of a particular drug for its binding site. Such binding of drugs to protein is usually reversible and is generally assumed to have very rapid rates of association and dissociation (milliseconds to microseconds).

The ratio of bound to unbound drug is governed by a number of factors which include the drug's $K_a$, its concentration, and the protein concentration. For most drugs used clinically in man, the degree of binding is essentially constant over the therapeutic concentration range. However, situations do occur where the level of drug approaches the molar concentrations of the binding protein and the unbound fraction varies over the plasma concentrations associated with therapy. An example is salicylic acid when used for the treatment of arthritis (or in cases of overdose). In animal studies, where relatively larger doses are often administered than in humans, concentration-dependent plasma binding is more frequent [20].

c) Distribution:

From the blood the drug may be distributed to various extravascular "compartments" of the body such as the extracellular fluid, different organs and tissues. Plasma protein and other binding sites influence tissue distribution of a drug; this is expressed quantitatively as $V_d$, the apparent volume of distribution. $V_d$ is expressed in units of volume but is not necessarily significant with respect to real volumes. A low $V_d$ indicates high plasma concentrations and likely reflects a high degree of plasma protein binding. A large $V_d$ can be hundreds of litres, and indicates high concentrations in tissue with respect to blood; this evidently reflects the degree of tissue binding. However, the problem of directly assessing tissue binding and
relating it to the overall distribution of a drug has limited investigations of this area. It is important to remember such factors since there is a possibility that tissue binding saturates with increasing dose, especially after large doses and during the distribution phase following intravenous administration of basic drugs [21]. Binding of drugs to tissue constituents has not been extensively investigated and the nature of the responsible macromolecules is largely unknown. Adipose tissue along with soluble and structural proteins and other components (nucleic acids, polypeptides, and polysaccharides) all have the capacity to reversibly bind a variety of compounds. However, studies of the mechanisms involved, and other characteristics of the phenomenon are hampered by the difficulties in measuring binding in the intact animal [20].

Accumulation of a drug in tissue depends not only on the extent to which it is bound to tissue components, but also on a drug's lipid solubility. The penetration of weakly acidic or basic drugs into cells depends on extracellular pH which is quite constant in the circulation. The rate of distribution from the circulation to the tissues of each organ will be determined not only by these characteristics but also by the blood flow to the organ. It is generally believed that blood flow is the rate-limiting step in distribution. However, distribution depends on the chemical composition of each tissue, and physicochemical properties of the drug such as pKₐ. Various types of drug may have preferred sites of accumulation in tissues.

This partitioning from the blood into the rest of the body is usually reversible, so that eventually *equilibrium distribution* can be achieved. Although the concentrations in blood and tissues are rarely equal, they are in equilibrium, and a
change in either will cause a redistribution. After an intravenous bolus dose of a drug it takes time for distribution to occur and because of drug elimination, steady-state conditions are not maintained. The time required to achieve distribution then depends not only on plasma protein and tissue binding but on the perfusion of the various organs of the body by the blood, the lipophilicity of the drug, and the clearance of drug from the blood. In this case the $V_d$ will change as drug moves from blood to tissue, finally reaching a "pseudo-distribution equilibrium". For most drugs, $V_d$ at steady-state and $V_d$ at pseudo-distribution equilibrium are within 10% of each other. When the difference is large, the more useful value is often the one obtained at pseudo-distribution equilibrium. This applies to all multicompartment models in which elimination occurs from a central compartment [22].

d) Elimination of drug from the body:

Metabolism and excretion are generally unidirectional processes which decrease the amount of available drug in the circulation (as it is either removed or converted to metabolites). Excretion may occur via urine, bile, and/or milk. As soon as equilibrium distribution is achieved, blood concentrations generally diminish by first-order kinetic processes (i.e. linear relationship between blood concentration and rate of elimination).

The plasma elimination half life of a drug, $T_{1/2}$ (the amount of time required to reduce the concentration of drug in the plasma by half), is arguably the most revealing pharmacokinetic property. This parameter, however, is only secondary and a function of two primary parameters - $V_d$ and clearance (rate of elimination from the body).
Dividing clearance by $V_d$ gives the elimination rate constant, $K$. After a bolus intravenous dose, $V_d$ does not remain constant with time and so the elimination rate constant $K$ also varies with time. As $V_d$ increases, it approaches a limiting value, thus the $K$ value decreases to a limiting value called the "terminal" rate constant. Its value and that of its corresponding $T_{1/2}$ are the numbers usually referred to in the literature [22].

e) Lipid solubility and pharmacokinetics:

The pharmacokinetic behavior of a molecule can be well defined in terms of absorption, clearance, and volume of distribution. These depend upon the physicochemical properties of the drug molecule. Lipophilicity at physiological pH is perhaps the most important [23]. The standard measure of lipophilicity is the partition coefficient ($p$) measured as solubility in water versus octanol, and usually expressed as log $p$. Lipophilicity is increasingly measured by means of indirect methods such as reverse phase liquid chromatography. This method is clean, rapid, and leads to useful structure-activity relationship data especially within structurally homologous series. However, log $p$ does not always reflect relative solubility under physiological conditions due to difficulties solubilizing some drugs at certain pH's and temperatures. A new variable, log D [24], allows for description of effective lipophilicity of a molecule at a given pH (e.g. physiological pH) and temperature and is probably a more useful unit of measurement than log $p$. Log D can be determined using buffered aqueous phases or calculated from $p$ as follows:
for organic bases: \( \log D = \log p - \log[1 + \text{antilog}(pK_a - pH)] \)

for organic acids: \( \log D = \log p - \log[1 + \text{antilog}(pH - pK_a)] \)

f) Compartmental systems of pharmacokinetic modeling:

Representation of pharmacokinetic data by a compartmental system was first considered in the 1930's [25,26]. A compartment can be defined as an anatomical region in which a drug is uniformly distributed. Usually it does not have an anatomical or physiological counterpart but may, e.g. plasma. The central compartment represents blood and highly perfused tissues. In a multi-compartmental model the peripheral compartments represent tissue with lower blood flow.

In the compartmental model of drug distribution kinetics, the one-compartment model is represented as plasma-time curves with first-order kinetics. For an intravenous dose the amount of drug eliminated from the body will equal the dose administered; therefore clearance (CL) = \( V_d \times K \). If the plasma-time curve does not show first-order kinetics (i.e. shows a nonlinear log plasma drug concentration vs. time curve relationship), a multi-compartment model may be used to describe the kinetics of distribution. In the two-compartment model the log concentration-vs-time curves are biphasic. These are termed the alpha and beta phases, or \( \lambda_2 \) and \( \lambda_1 \). Each of these "disposition phases" has its own \( T_{1/2} \) value. Generally, \( T_{1/2} \) values reported for drugs refer to the beta phase half-life.
g) Usefulness of pharmacokinetic data:

This thesis is primarily concerned with the distribution stage of drug kinetics; specifically tissue distribution. Tissue levels are a valuable piece of information for clinicians since together with the blood concentration they give an accurate method of calculating total amount of drug remaining in the body at any time. One can correlate tissue concentration data with pharmacological response and estimate onset and offset of action. Determining maximum tissue concentration during the alpha phase in organs where the drug is pharmacologically active may help predict toxicity.

For most drugs it is desirable to have specificity of action and this may depend on distribution. This process can involve competition between the target organ and other tissues where affinity may be higher. This could result in less therapeutic effect and possibly unwanted side effects. Conversely, if drug concentration at the target organ is too high before steady state levels are reached, toxic effects could manifest themselves before any therapeutic effects are obtained. This is particularly true for drugs that affect the heart and have a small therapeutic margin. If the dose is lowered to prevent toxic effects during the alpha phase, the steady state concentration may then be below the therapeutic level.

The rapidity with which a drug is transported to the target organ is the prime determinant of onset of action. Since the heart is highly perfused and RSD compounds are lipophilic weak bases we expected no difficulties in achieving therapeutic concentrations in the target organ. Minimal penetration across the blood-brain barrier would limit possible central nervous system side effects. However, since the tested drugs are \( \kappa \)-agonists, significant binding in the brain was expected.
Skeletal muscle data gave insight into accumulation in muscle beds. Storage in muscle and adipose tissue, if great enough, can be a major cause of decrease in availability for more perfused tissues and be responsible for maintaining plasma levels over long periods of time (which could lead to toxicity after chronic use). In humans one cannot readily determine tissue levels of drugs by direct sampling but there are models and mathematical methods for estimating this. Measurement can be done in animals however, and this can be used to predict distribution in man and be correlated with mathematical predictions.

III. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:

High performance (or high pressure) liquid chromatography (HPLC) is a quantitative analytical method for measurement of the concentration of compounds in solution. These analyte solutions may be homogenates of tissue or plasma samples that have undergone appropriate chemical treatments to reduce interfering substance content. Liquid chromatographic techniques complement gas chromatography by their application to the separation of non-volatile substances, and are particularly suitable for separations of compounds that are large, highly polar, thermally unstable, and/or easily ionisable in solution. Optimal rates of solvent flow require conditions of high pressure due to the higher viscosity of liquids as compared to gases.

An HPLC system is composed of an optional automated injector system (useful for analyzing large sample numbers), a solvent pump (either of constant-pressure or
constant-flow type), the column, a detector (can be ultraviolet, electrochemical, fluorescence or other), and a chart recorder and/or computerized data system. Individual substances show up on the chromatographic record as peaks rising above the solvent baseline.

A chromatography system is tuned so that peaks are as narrow as possible and well separated from each other. With HPLC the most important determinants of peak profile are the packed bed within the column and the solvent (eluent) composition. The column packing material is usually composed of a chemically modified particulate silica gel. The parent silica particles are micrometers in diameter (typically 3-7 μm) and have on their surfaces silanol groups (Si-OH) that can be reacted with any number of compounds to form a chemically modified surface that can specifically retain the compound(s) of interest (the analyte). The porous nature of the packing material may also make it possible to separate on the basis of size and increases the surface capacity of the column. The size of the gel particles is most important in determining separation efficiency. The smaller the particles, the larger the plate number (a plate being that fraction of the column length in which an analyte molecule goes through one cycle of attraction and removal). Increasing plate number (within limits set by pressure and time) minimizes peak dispersion. Solvent composition (organic content, added buffer salts or surfactants) also affects peak profile and relative retention of analytes.

HPLC separations often use several mechanisms of retention with any one column packing chemistry. Molecules of analyte can be retained by reactive silanol groups, the polar components of the eluent can be adsorbed creating a thin organic
layer on the surface of the gel beads that will allow for separation on the basis of polarity (liquid-liquid partition chromatography), charged groups can be attached either covalently or by association with the silanols to allow for ion-exchange chromatography, and other non-bonded interactions with alkylated surfaces can also be used to modify relative retention. The system used for the studies in this thesis was primarily operating in the mode known as reverse-phase chromatography.

Reverse-phase chromatography is so named because rather than the "normal-phase" method of using an organic eluent and a polar stationary phase (the reactive groups on the gel particles), it utilizes aqueous eluents containing a proportion of organic solvent and a non-polar (hydrocarbon-bonded) stationary phase. The organic component of the eluent is retained by the beads to form an organic-rich layer at the particle surface which has a high affinity for lipophilic compounds. In this way transport of the most polar compounds proceeds with little retardation, and the more lipophilic compounds are retained to give extended elution times. This technique is particularly useful for the separation of non-polar compounds from primarily aqueous solutions which may have a high level of polar impurities derived from tissues or biological fluids.

The quantitative aspect of the analysis comes from measurement of the peaks. Peak height may be used to estimate analyte amounts on the assumption of constant height:area ratios but integration of area under the peak is the fundamental quantitative datum for HPLC quantitation. The area or height values are proportional to the amount of analyte passed through the column. All samples should contain an internal standard, which is a substance that is as chemically similar to the analyte as
possible so that both will undergo extraction procedures with equal effectiveness. A known concentration of internal standard is used with each sample. Calculations can be performed using a standard curve of peak area ratio (analyte:standard) vs. concentration of analyte, or using the equation \( C_x = (P_x/P_s)C_s \) where \( C_s \) is the concentration of the internal standard, \( P_s \) is peak height (or area) of the standard, and \( P_x \) is peak height (or area) of the analyte. This latter method is performed following a duplicate run of sample with a standard sample run before and after.

**IV. HYPOTHESIS AND OBJECTIVES:**

The hypothesis put forward in this experiment was that drug disposition in the early distribution phase would mimic the time course of the pharmacodynamics. To examine this, a profile of concentration-vs.-time for blood and four different tissues was constructed using the rat. The objective is to demonstrate that the rise and fall of drug concentration in the target organ and the blood would follow the same time course as onset and offset of therapeutic effect as observed in the rat in separate studies using this drug.
MATERIALS AND METHODS

Materials:

PD123,497 and the internal standard (a structural analogue of PD123,497) were synthesized by and obtained from Cindy Longley and Anne Morrison of Rhythm Search Developments (Vancouver, B.C., Canada). HPLC grade MeCN, MeOH, and acetone were obtained from Fisher Scientific (Nepean, Ont., Canada). Methyl-t-butyl ether was obtained from BDH, and various non-organic reagents were used of ACS standard and obtained from the usual laboratory supply firms.

Protocol:

Eighteen male, Sprague-Dawley rats weighing from 300 to 450 grams were randomly assigned to one of six groups (n=3). PD123,497 (8 μmol/kg) was administered i.v. (tail vein) and rats were sacrificed by decapitation at t=15 sec., 30 sec., 1 min., 2 min., 5 min., or 10 min. post-injection. Brain, heart, liver and a skeletal muscle sample taken from the right hind leg were removed, rinsed in cold saline (0.9% w/v), and frozen at -20°C. Blood (8 - 13 mL) was collected from the neck area, mixed with a few drops of heparin (1000 i.u./mL), and diluted with saline. This mixture was homogenized and frozen at -20°C.

Extraction:

There are many methods of chemical extraction used to purify samples for analysis. Several of these were tried and rejected due to poor recoveries or technical
difficulties. The procedure described below is rather complex, but was necessary for maximum recovery of drugs and reproducibility of results.

Tissues were thawed, weighed, and homogenized in saline. Blood homogenates were thawed and stirred prior to extraction. Each homogenate was aliquotted into three 2.5 mL samples. To each sample was added 2.2 nmol/mL internal standard; samples were lightly vortexed, then 3 mL acetone was added to each. After vigorous vortexing to a uniform texture, samples were let stand for 15 - 30 minutes then centrifuged at 5500 rpm for approximately 15 minutes. A fixed volume of supernatant was removed and the pellet discarded. To the supernatant was added 0.1 volume of NaH₂PO₄ buffer (0.1 M, pH=9), then 5 mL of MtBE. Tubes were mixed on a Labquake mixer (LabIndustries, Berkeley, California, USA) for 10 minutes. The organic fraction was removed and two more washes with 2 mL MtBE were performed on the aqueous fraction. After the last wash the aqueous fractions were discarded. A back extraction was performed on the organic fractions using 0.4 M sulfuric acid: three washes of 5, 2, and 2 mL respectively. The aqueous fraction was collected this time and after the final wash the organic fractions were discarded. The aqueous fractions were then neutralized by adding solid sodium carbonate until pH paper indicated a pH between 8 and 9. The MtBE washes were repeated as above and the aqueous fractions discarded. The organic fractions were evaporated under nitrogen gas in a Reacti-Therm heating module (Pierce Chemical Co., Rockford, Illinois, USA) set on low heat. Dry samples were reconstituted by first dissolving in 50 µL ethanol, sonicating, then adding 450 µL distilled water so that total sample volume = 500 µL.
Recovery of drug during extraction:

To determine extraction efficiency blank homogenate solutions from blood and the four tissues studied were spiked with standard solutions of PD123,497 (0.6, 1.3, 2.5, or 5.1 nmoles/mL) and internal standard (2.2 nmoles/mL). Dividing the peak area of an extracted sample by that of the unextracted standard solution gave percent recovery of that drug at a particular concentration.

Quantitative Analysis:

Samples were analyzed using high performance liquid chromatography. Injection volume was 20 µL. The system was composed of a WISP 710B automated injector (Waters, Milford, Massachusetts, USA), a twin piston Beckman 100A pump (Beckman Instruments, CA, USA), a Model 441 Waters Associates 215 nm fixed wavelength UV detector, a Rikadenki model B104 chart recorder (Rikadenki Kogyo Co., Tokyo, Japan), an SGE cyanophase 10GLC4 precolumn (10mm x 4mm, 5 micron particle size, 80 angstrom pore size; Fisher Scientific), and an SGE cyanophase column (100mm x 4mm, 80 angstrom pore size, 5 micron particle size; Fisher Scientific). Data was analyzed with an Apple Ile computer using the Chromatochart program (Interactive Microware Inc., State College, PA, USA). The mobile phase was composed of 45% MeCN, 16% MeOH, and 8.5% 0.15M ammonium acetate buffer (pH=7) and degassed in situ by helium at ambient temperature. Samples were eluted isocratically.
Calibration curves:

Calibration curves were made by plotting peak area ratio (analyte:standard) vs. concentration of PD123,497. A curve was made for unextracted and extracted standard solutions. A separate graph of the two curves was constructed for each tissue and for blood.

Concentration-vs-time graphs:

For each tissue, concentration at each time point was determined. The peak height ratio of the three aliquots of each sample were averaged and the concentration (nmoles/mL) was determined by extrapolating from the appropriate calibration curve (extracted sample curve). Tissue concentration (nmoles/g tissue) was determined by multiplying by the appropriate variables (see Appendix 1 for calculations), then the three concentrations for each rat were averaged to obtain a mean concentration for each tissue at each time point.
RESULTS

Extractions:

The recoveries of PD123,497 and internal standard for blood and the four tissues are shown in Table 2. The brain and liver homogenates yielded the lowest recovery of PD123,497 (77 ± 0.7% and 77 ± 3.8% respectively) and the highest recovery was that of internal standard from brain homogenate (90 ± 0.9%).

Calibration curves:

Calibration curves for the various tissues and blood and their regression coefficients (r) are shown in Figure 1 (a - e). Regression analysis showed all calibration curves to be linear (of the equation y = mx + b). Concentration in unknown samples was determined using the extracted sample data on the appropriate calibration curve.

Area under the peak:

Variance within days: For each day's analyses two runs of standard solution were performed. The coefficient of variance for these peak areas for any one standard solution was less than 5%.

Variance between days: The same stock solution of standard was used for several days. The coefficient of variance for average peak area (n=2) over a series of days (n=5 or 6) was less than 5%. 
<table>
<thead>
<tr>
<th></th>
<th>Mean Recovery(%) ± SEM</th>
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<tbody>
<tr>
<td>int.standard</td>
<td></td>
</tr>
<tr>
<td>PD123,497</td>
<td></td>
</tr>
<tr>
<td>liver</td>
<td>87 ± 1.1</td>
</tr>
<tr>
<td>heart</td>
<td>88 ± 1.5</td>
</tr>
<tr>
<td>sk. muscle</td>
<td>88 ± 1.7</td>
</tr>
<tr>
<td>brain</td>
<td>90 ± 0.9</td>
</tr>
<tr>
<td>blood</td>
<td>87 ± 1.4</td>
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**TABLE 2:** Recovery of PD123,497 and internal standard by chemical extraction:

The % recovery of PD123,497 and internal standard following extraction using the method described is shown. Blank homogenates were spiked with 0.6, 1.3, 2.5, or 5.1 nmol/mL PD123,497 and 2.2 nmol/mL internal standard before liquid extraction with MtBE and sulfuric acid. Each number represents the mean and SEM of the recoveries for 12 samples (n=3 at each of four concentrations of PD123,497).
FIGURE 1: Calibration Curves for Blood and Tissues: Blood and tissue homogenates were spiked with 2.2 nmoles/mL internal standard and either 0.6, 1.3, 2.5, or 5.1 nmoles/mL PD123,497. After extraction using the method described peak area ratios were plotted against concentration of PD123,497 to give a calibration curve (●). These were used to calculate concentration of PD123,497 in unknown samples by extrapolation using peak area ratio. Curves were also constructed for unextracted standard solutions of PD123,497 and internal standard (Δ). These were used to determine extraction efficiency. Regression coefficients (r) are given for all curves.
**LIVER**

- ● standards
- △ extracted

![Graph D](image)

**BLOOD**

- ● standards
- △ extracted

![Graph E](image)
Pharmacokinetic data:

Figure 2 (a - d) shows concentration-vs-time graphs for brain, heart, skeletal muscle and liver as compared to blood. Data points are shown with error bars indicating standard error of the mean (SEM). Figure 3 shows all four tissues and blood on one graph so that a complete time profile of the drug's course through the body may be observed.

The drug distributed rapidly into the well perfused tissues. In fact, the concentration in the heart after only 15 seconds was several times higher than that in the blood. The heart and brain exhibited a triphasic distribution profile with a minimum concentration occurring at the 30 second time point and then increasing again before dropping off to a characteristic beta-phase profile. This coincided with the apparent transition from alpha-phase to beta-phase in the blood. This triphasic tissue profile was also seen in skeletal muscle but the first minimum did not occur until around the 60 second time point and the valley was of a broader shape. The liver exhibited a slow increase in concentration over time and at the 10 minute mark was still increasing.

The rise in concentration in the liver mirrored the decrease in concentration in the heart. Brain concentration was still increasing with liver concentration until the 2 minute mark and then it dropped rapidly, leveling off at the 5 minute mark. Skeletal muscle concentration did not change much after reaching a second maximum at around 2 minutes.
Figure 2: Concentration vs. time graphs for blood and tissue: The concentration of PD123,497 in blood (●) and tissue (Δ) at various times following bolus intravenous injection of 8 μmol/kg is shown. Each point is the mean tissue concentration for three animals. For each animal, three aliquots of homogenate were analyzed and the peak area ratios were averaged before extrapolation on a calibration curve to determine drug concentration. Error bars represent standard error of the mean (SEM). Points without error bars have SEM's that are too small to be seen on the scale of the graph. A: blood and brain, B: blood and heart, C: blood and liver, D: blood and skeletal muscle.
A

BRAIN

- blood
- brain

conc. (nmoles/g tissue or mL blood)

0 60 120 180 240 300 360 420 480 540 600

time after injection (seconds)

HEART

- blood
- heart

conc. (nmoles/g tissue or mL blood)

0 60 120 180 240 300 360 420 480 540 600

time after injection (seconds)
**C**

**LIVER**

- **blood**
- **liver**

<table>
<thead>
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<th>Conc. (nmoles/g tissue or mL blood)</th>
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<td>0</td>
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Time after injection (seconds)

**SKELETAL MUSCLE**

- **blood**
- **sk.musc.**

<table>
<thead>
<tr>
<th>Conc. (nmoles/g tissue or mL blood)</th>
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Time after injection (seconds)
Figure 3: Disposition of PD123,497

The distribution of PD123,497 in blood and tissues following a bolus intravenous dose of 8 μmol/kg is shown. The peak area ratios of three aliquots of a sample homogenate were averaged and this was extrapolated on a calibration curve to obtain a tissue concentration. Each point on this graph represents the average tissue concentration for each of three animals. (for error bars see figure 2)
DISCUSSION

The distribution of PD123,497 in the blood showed a standard biphasic profile as described by a two-compartment model of pharmacokinetics (see figure 3). The two phases, representing two different rates of removal from the blood, are called the alpha and beta phase respectively. The alpha phase generally represents redistribution into peripheral compartments and the beta phase represents clearance from the blood by metabolism and/or elimination mechanisms.

Concentration-vs-time profiles for PD123,497 in the brain, heart, and skeletal muscle were relatively similar with differences mostly in the time course of events (see figure 3). The apparent transient decrease in concentration seen in heart and brain was lowest at 30 seconds and concentration was rising rapidly at 60 seconds whereas in skeletal muscle concentration was still minimal at this point. This is not surprising since skeletal muscle is not as well perfused as the other two organs and so one would expect slower rates of drug uptake and elimination. Although drug enters and exits skeletal muscle at a slower rate than the highly perfused tissues, a significant amount of drug is still getting in. It is necessary to keep in mind that the skeletal muscle data represents a small sample of the total skeletal muscle in the body of the rat. Although the concentration is lower than that of the other tissues, the actual amount of drug sequestered in the skeletal muscle is considerable given the amount of tissue it represents.

Free (unbound) drug on one side of a diffusible membrane will flow to the other side until concentration is equal on both sides of the membrane. Therefore, any
difference in the total amount of drug between blood and tissue reflects a difference in
the amount of bound drug at steady state (this does not hold for situations where drug
is actively transported across the membrane). If the drug concentration in a particular
tissue is lower than that of the blood, it means either the drug is bound in the blood
and not in the tissue, or at least bound to a lesser extent than in the blood. If the
concentration is higher in a particular tissue, this reflects a binding site in the tissue
with a higher affinity than binding sites in the blood. The tissue distribution of
PD123,497 reflected both such situations. In the heart, the concentration initially was
much higher than in the blood. This indicated a readily accessible binding site in the
heart with a higher affinity than that of the blood. In the brain, drug concentration was
initially lower than in the blood, so there may be a low affinity site in the brain or none
at all in which case the total amount of drug measured in the brain immediately after
injection reflected free drug. Again, this is assuming that the membrane is freely
diffusible and no active transport system is involved in crossing the membrane.

The liver is a highly perfused organ and one would expect the drug to obtain
maximum concentrations as rapidly as in other highly perfused organs such as the
brain and the heart. And since the liver is a major organ for metabolism, drug
concentration would likely begin to decrease soon after. The data for PD123,497
showed an extremely low concentration in the liver after the first 15 seconds when
concentrations in the heart and brain were very high. Even if there was no extraction
by the liver we would still expect a significant amount of drug due to the high rate of
perfusion. If this low concentration is due to rapid metabolism of drug by the liver, we
would expect the concentration of parent drug to decrease over time as blood
concentration falls; instead, after a short delay, the concentration starts to increase. This does not seem to indicate significant metabolism. Perhaps the time course over which data was collected was not sufficient to demonstrate the true profile of drug in the liver; it is not clear whether a maximum concentration was reached by 10 minutes. However, an explanation for this phenomenon is the presence of a storage depot within the liver, possibly the fat globules in hepatocytes. Drug entering the liver is at first rapidly extracted and so levels of drug in the tissue remain low. As time progresses, the drug molecules eventually make their way deeper into the cell where they are sequestered in the fat globules. Thus, concentration of drug in the whole organ would rise as drug is trapped in these depots, free from the metabolizing enzymes in the cytoplasm. This process would take time since an intracellular site is not rapidly accessible from the circulation, but it is saturable and eventually liver concentration would begin to decrease. This may explain why concentration in the liver is still rising while in other tissues it has reached a minimum.

In three of the tissues there was an interesting feature in the kinetics of PD123,497: a transient decrease in tissue concentration that occurred early in the time course of distribution. This unusual "negative peak" may have been an artifact but because it appeared in three different tissues (heart, brain, and skeletal muscle) it was worth examining. Between 15 and 30 seconds after injection, the concentration of drug in these three tissues decreased with a corresponding decrease in the blood concentration, but by 120 seconds the concentration in these tissues rose again even though blood concentration was still declining. At this time point we also saw the
concentration in the liver begin to rise rapidly. I have put forward a theory to explain these events.

It is possible that the data above reflects drug binding in a tissue that was not analyzed in this experiment. This "invisible" binding site competed with binding at other sites and was responsible for the transient decrease seen in the concentration-vs-time profiles. Note that initially most of the drug in tissue will be in the interstitial fluid, a readily accessible, high-capacity compartment. Although our unknown binding site is not as accessible to the drug as these extravascular sites in the other tissues, the binding affinity is higher and drug is soon preferentially sequestered here. Thus we see a rapid drop in concentration for blood, heart, liver, and skeletal muscle as drug is redistributed to the unknown binding site. This site is soon saturated but there is a second tissue binding site in the brain, heart, and skeletal muscle located intracellularly that is now preferentially sequestering the drug. This high-affinity site is not as readily accessible, thus the time delay. Drug is redistributed back to the heart, brain, and skeletal muscle. The result of this overall process is concentration-vs-time curves with two concentration maxima reflecting two separate binding sites: a low-affinity extracellular site, and a high affinity intracellular site. In a compartmental model, the central compartment would represent blood and the extracellular matrix, the unknown site would represent compartment two, and the brain, heart, and skeletal muscle would represent compartment three. Possible candidates for the unknown site are the lungs or the kidneys, both highly perfused tissues.

It is also possible that the unknown binding site described above is the gut and that PD123,497 undergoes enterohepatic circulation. In this case drug is rapidly
extracted by the liver into the bile which explains the rapid drop in concentration seen in the blood and other tissues during the first 30 seconds after injection, and the low concentration in the liver. The bile is delivered to the gut where it binds to some low affinity site. Upon saturation, blood levels stabilize and enough time has passed that the drug has penetrated higher affinity intracellular sites in the other tissues and is now preferentially sequestering here. It is also being stored in the liver and so concentration in all four tissues goes up. This is one possibility, however, further work is required to clarify the appearance of this transient decrease in concentration seen early in the time course of tissue distribution of PD123,497.

After saturation of the second, high-affinity site in the heart, brain, and skeletal muscle, concentrations declined. This drop occurred early in the heart (peak concentration at 60 seconds), later in the brain (peak concentration at 120 seconds), and latest in the skeletal muscle (peak concentration at 300 seconds). These differences reflect affinity of the binding site and of course, rate of perfusion of the tissue. The concentration maxima appear later in other tissues than in the heart and in this way PD123,497 is mimicking thiopental where cessation of therapeutic activity is also due to redistribution into other tissues and not elimination. Concentration of PD123,497 in the liver was still increasing at 600 seconds reflecting sequestration of drug. It is interesting to note that at this time point the liver samples run on the HPLC had an extra peak on the chromatograph that could represent a metabolite.

When the actual amount of drug in the five areas is added up, there is still a large percentage of the administered dose unaccounted for. This is possibly due to uptake into body fat. PD123,497 is highly lipophilic and there is no reason to believe
there would be any difficulty crossing lipocyte membranes. Rate of uptake would be slow due to the poor rate of perfusion in fatty tissue, but this site represents a high-capacity depot that should not be overlooked.

So how does all of this data relate to pharmacological action? Several antiarrhythmic screens of PD123,497 have been performed in different animal species so far. Many of these tests involved bolus intravenous doses of 8 µmol/kg - the same protocol that was used to obtain the pharmacokinetic data presented here. It is therefore possible to directly compare the data.

Two dose-response curves were constructed for the effects of PD123,497 on heart rate at 1 minute and 8 minutes after injection in the rat [27]. After 1 minute post-injection (bolus i.v.) of 8 µmol/kg PD123,497 there was a drop in heart rate from 415 bpm (pre-injection) to 305 bpm. Eight minutes later the heart rate was back up to 385 bpm. This correlated well with my data which showed a higher concentration in the heart at 1 minute than at 8 minutes post-injection.

The same experiment in the baboon [27] yielded similar results. One minute after bolus i.v. injection of 8µmol/kg PD123,497 there was a 26% decrease in heart rate from control, whereas at 8 minutes there was only an 8% decrease. The data presented here for the rat does show that at 8 minutes concentration in the heart is lower than at 1 minute post-injection, however the difference is much greater than 18%. The results are still appropriate however when one considers that the baboon is a much larger animal than the rat and would therefore be expected to have slower kinetics of uptake and elimination (due in part to a slower heart rate). The slopes of the beta-phase portion of the heart curve may be different for the two species, but the
fact appears to remain that concentration in the heart is declining at this time point in the rat and the baboon.

Another experiment in rats measured the effect of PD123,497 on heart rate and blood pressure at frequent intervals after a bolus intravenous dose of 8 μmol/kg [27]. These results correlated very well with the heart and blood concentration data presented here (see Figure 4).

In two of three dogs (see figure 5), bolus i.v. doses of 1.0, 2.0, and 4.0 μmol/kg PD123,497 produced a greater percent decrease in conduction velocity 2.5 minutes after injection than at 5 minutes after injection [27] (the exception being dog #1 at the two lower doses where there was no significant change). Although the doses were lower than those used to obtain the rat pharmacokinetic data, it still correlated well. We might expect the time course of elimination from the heart to be slightly longer in the dog than in the rat, but it would seem that concentration is decreasing in the dog heart by five minutes and thus following the same profile as seen in the rat.

Besides relating to pharmacological response, concentration-vs.-time curves can also provide useful information with regards to potential toxicity. Therapeutic concentrations must be maintained in the beta phase of distribution, yet the alpha-phase concentrations are often much higher. If concentrations in the alpha phase are high enough in certain tissues, toxicity may result before any therapeutic benefit is achieved. For example, PD123,497 exerts its primary therapeutic activity in the heart by slowing conduction velocity and increasing the refractory period. This antiarrhythmic effect can easily become pro-arrhythmic and the therapeutic margin of AA's is notoriously low. The pharmacokinetic data for PD123,497 shows a very high
Figure 4: Concentration vs. Effect: The relationship between concentration and pharmacological effect is demonstrated in the following graphs. Heart and blood concentration data were obtained by the methods described. Heart rate and blood pressure data are from pharmacological screens performed by other researchers at Rhythm Search Developments. The data are presented together to demonstrate the strong correlation between the results of these different studies. A: heart rate, B: blood pressure.
PD123,497 and Conduction Velocity
Canine left ventricle

---

Dog #1
---

Dog #2
---

Dog #3

Figure 5: Effect of PD123,497 on Conduction Velocity in the canine heart.

This data, from work done by fellow researchers at Rhythm Search Developments (unpublished), shows the effect of three bolus intravenous doses of PD123,497 on conduction velocity at 2.5 and 5.0 minutes after each dose. This data correlates with the findings presented here that concentration of PD123,497 in the heart is greater 2.5 minutes after a bolus i.v. dose than at 5.0 minutes after.
initial concentration in the heart, higher even than in the blood. Not only does this suggest possible alpha-phase toxicity but it is another good example of why tissue distribution studies are so important. A clinician relying only on plasma or whole blood concentration to monitor dosage would have to be aware of the fact that these figures underestimate the concentration achieved in the target organ and do not indicate the dangerously high initial concentrations of the alpha phase following i.v. administration.

Tissue distribution data is also helpful in drug development. Along with various pharmacological screens for activity and toxicity, pharmacokinetic profiles can provide useful information for evaluating the clinical potential of test compounds. The kinetics of PD123,497 indicate that after reaching peak concentration in the heart at 1 minute, a peak brain concentration is achieved at 2 minutes. This implies that as cardiovascular effects are wearing off, significant CNS effects may appear, a likely event considering these drugs were developed originally as kappa-opioid agonists. This is an important consideration when evaluating this drug as a potential prototype for future research. Cardiovascular effects are warranted in the treatment of cardiac arrhythmias; CNS effects are not. The slow buildup of PD123,497 in the liver may also be of some concern if this drug proves to be hepatotoxic.
CONCLUSION

The method presented here for extraction and quantitative analysis of PD123,497, a novel kappa-opioid agonist, was demonstrated to be effective in terms of recovery and reproducibility. Furthermore, as a tool for the pharmacokinetic analysis of PD123,497 distribution in the rat, it produced results that painted a distinct picture of the time course of the drug’s travels through the whole animal. The results also correlated well with previous pharmacological screens using the same drug in various animal models.

The data showed that PD123,497 was rapidly distributed into the heart and has a potential for alpha-phase toxicity. Termination of therapeutic effect was due to redistribution to other tissues and not metabolism or elimination. An unusual triphasic curve observed in three of the tissues suggests another significant compartment for PD123,497 distribution not represented in the samples analyzed here. There may be a significant capacity for storage in muscle and adipose tissue. Possible storage in the liver could result in hepatotoxicity. Finally, a significant amount of drug was distributed to the brain, suggesting a potential for CNS effects.

The hypothesis put forward in the Introduction was supported by this work. Specifically, the onset and offset of therapeutic effects correlated well with the time course of rise and fall of concentration in the blood and heart.
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   **160**: 470 (1962)


**BIBLIOGRAPHY**


APPENDIX I

Calculations to determine tissue and blood concentration:

1. using peak area ratio (average of three aliquots), concentration of PD123,497 was extrapolated from the appropriate graph:

\[
\text{concentration of PD123,497 (nmol/mL)} \times \frac{\text{volume of sample (0.5 mL)}}{\text{volume of homogenate (2.5 mL)}} \times \frac{\text{original volume of weight of tissue sample or volume of homogenate (mL)}}{\text{blood taken from rat (g or mL)}} = \text{concentration of PD123,497 in original sample (nmol/g tissue or mL blood)}
\]