

Power Spectral Analysis of Heart Rate Variability in Rats as a Quantitative Tool in the PK-PD Analysis of the Parasympatholytic Activity of Atropine

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Purpose. To utilize power spectral analysis (PSA) of heart rate variability (HRV) as a pharmacodynamic (PD) measure of atropine parasympathetic effect, and to model the kinetics of action.

Methods. Heart rate data was collected following atropine administration to rats and was analyzed off-line for high frequency peaks by PSA of HRV as a measure of parasympathetic tone. A temporal cumulative approach (TCA) detected transient changes in parasympathetic activity. The pharmacokinetics (PK) was analyzed and linked to both direct and indirect PK-PD models.

Results. TCA enabled a quantitative measure of atropine parasympathetic activity. A simultaneous fit of the indirect PK-PD model to the experimental data of all three atropine doses successfully captured the experimental data.

Conclusions. TCA can be used as a quantitative measure of parasympathetic tone. Our work has established a preclinical model to investigate the kinetics of drug action on the autonomic nervous system.

KEY WORDS: atropine; pharmacokinetics; pharmacodynamics; indirect response model; power spectral analysis; heart rate variability.

INTRODUCTION

Although cholinergic drugs are commonly used for treatment of various pathophysiological conditions ranging from Alzheimer and Parkinson diseases (1,2) to the prevention of organophosphate toxicity (3), their kinetics of action on the

autonomic nervous system (ANS) is not clearly defined. The major obstacle in addressing this issue is to quantify *in vivo* the ANS activity and temporal changes in magnitude of response to the drug action. The complexity evolves from the nature of the ANS, which responds simultaneously to many internal and external stimuli, and the constant balancing activity of different auto-regulatory systems, including the sympathetic and parasympathetic branches. As a result, when measuring autonomic activity, "noisy" data is obtained.

Over the past two decades, power spectral analysis (PSA) of heart rate variability (HRV) has been utilized as an indirect measurement of ANS activity (4,5). Following this frequency analysis, three major frequency bands are isolated: bands of high (HF), low (LF), and very-low frequency (VLF). It has been accepted that the HF component directly represents the activity of the parasympathetic system (6–8), whereas the LF and the VLF reflect combined activities of the sympathetic and the parasympathetic systems (6,9).

In this work we have focused on the parasympathetic action of atropine as a model anticholinergic drug. Although the HF peak obtained by the PSA of HRV in a rat model is identifiable and distinct, the magnitude of the HF peaks is considerably unstable even when consecutive time epochs of 2 min are compared (10). To overcome this serious impediment, we applied a new methodology based on the cumulative approach of the HF component of the PSA of HRV (10). The approach, described here, enables the identification of notable temporal changes in autonomic tone, thus providing a means to quantify the magnitude of response of the ANS at different times following drug administration. Consequently, we have linked the drug blood concentration-time data with the magnitude of the response using an indirect response pharmacodynamic (PD) model. This integrated pharmacokinetic-pharmacodynamic (PK-PD) model enables a description of the kinetics of action of atropine in reducing the parasympathetic tone at different doses.

MATERIALS AND METHODS

Animals

Male Sabra rats weighing 300–350 g were housed separately in plastic cages and were maintained at a 12-h light/dark cycle with food and water available *ad libitum*. The project adhered to the Principles of Laboratory Animal Care, a National Institutes of Health publication.

Transmitter Implantation

To minimize the stress involved with data collection, a telemetric monitoring system was implanted in the peritoneal cavity under anesthesia (50 mg/kg ketamine and 10 mg/kg xylazine, i.p.). The telemetric device is a small-sized (2-inch sq.), two-lead ECG radio frequency transmitter (TCA-F40, D.S.I., St. Paul, MN). The leads were tunneled subcutaneously to their positions at the right acrotrapezoidal muscle and the left gluteus muscle. PD experiments took place after a recovery period of at least 1 week.

Catheter Implantation

To enable both i.v. drug administration and withdrawal of blood samples, an indwelling polyethylene catheter was

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ABBREVIATIONS: TCA, temporal cumulative approach; pupm, power units per minute; AIC, akaike criterion for model selection; SC, Schwarz criterion for model selection; C_p , drug concentration in central compartment; X_p , drug amount in central compartment; X_t , drug amount in peripheral compartment; k_{10} , first order rate constant of drug elimination from the body; k_{12} , first order rate constant of drug transfer from central to peripheral compartment; k_{21} , first order rate constant of drug transfer from peripheral to central compartment; V , volume of distribution; K_{in} , production rate of the measured response (zero order kinetics); K_{out} , elimination rate of the response (first order kinetics); R , the magnitude of the measured response; I_{max} , maximal inhibition of K_{in} ; IC_{50} , drug concentration that produces 50% of I_{max} ; E_{max} , maximal drug effect; EC_{50} , drug concentration that produces 50% of E_{max} ; n , shape factor.

implanted in the right jugular vein under light ether anesthesia and was filled with heparinized saline solution (20 I.U./ml).

Compounds

Atropine sulfate was obtained from Sigma Chemical Co. (St. Louis, MO). [³H]-Quinuclidinyl-benzylate ([³H]-QNB) with specific activity of 50 Ci mmol⁻¹ was purchased from Amersham International (Buckinghamshire, UK).

Experimental Design

To eliminate the stress effect on the ANS, serial blood sampling was performed separately on different days. To assess the effect of different doses of atropine on ANS activity, the rats received the following drug treatments: 0.5, 1.0, or 2.0 mg/kg atropine sulfate solutions, or vehicle (normal saline). Each treatment was given as an i.v. bolus injection (0.3 ml over 3 min).

Cardiac Signal Acquisition System

Drug effects on the ANS were collected and analyzed using the temporal cumulative PSA of HRV method, described in detail elsewhere (10,11). Briefly, transmitted ECG data of the rats was acquired online, and the R-R interval (RRI) was recorded. Data artifacts were corrected offline (12) and records were divided into consecutive 2-min epochs that used autoregressive PSA following a Levinson algorithm (13). Two distinct peaks were obtained by the HRV power spectrum: VLF peak (0.01–0.20 Hz) and HF peak (range 1.35–2.65 Hz).

The Temporal Cumulative Approach

The cumulative plot was constructed by summing the HF (parasympathetic) peak vs. time plot. This plot was analyzed by a stepwise linear regression procedure (based on “least squares method”) that isolated time segments with consistent parasympathetic tone (11). The length of these linear segments was determined using the minimal error sum of squares (ESS) with a computerized procedure that searched for the minimal number of linear segments that could describe the cumulative plot. The minimal number of segments was determined when an additional vertex point reduced the sum of ESSs by less than 10%. The intensity of the parasympathetic tone at each time segment is defined by the slope in units of power units per minute (pupm).

Drug Concentration in Plasma and Pharmacokinetic Analysis

Following administration of atropine at doses of either 0.5, 1, or 2 mg/kg to rats, serial blood samples (~0.3 ml) were obtained at 5, 10, 15, 30, 60, 120, and 240 min. Serum was separated by centrifugation and stored at -70°C pending analysis. Serum atropine concentrations were determined by radioreceptor assay with [³H]-QNB according to the method of Aaltonen (14). The outcomes of the assay were linear for serum atropine concentrations between 0.4 to 40 µg/mL. The inter- and intra-day coefficients of variations were below 1% and 0.05%, respectively. The lower limit of quantification was 1.0 ng/ml.

Pharmacokinetic Analysis

A two-compartment model following bolus input was fitted simultaneously to all the concentration-time data sets using ADAPT II Pharmacokinetic/Pharmacodynamic Systems Analysis Software (Biomedical Simulations Resource, Los Angeles, CA), and the major PK parameters were determined (15). The differential equations used to describe the PK model were as follows:

$$dX_p/dt = -(k_{10} + k_{12}) \cdot X_p + k_{21} \cdot X_t \quad (1)$$

$$dX_t/dt = k_{12} \cdot X_p - k_{21} \cdot X_t \quad (2)$$

where X_p and X_t are the drug amounts at the central and peripheral compartments (respectively), and the rate constants are: k_{10} elimination from the body, k_{12} transfer from the central to the peripheral compartment, and k_{21} transfer from the peripheral to the central compartment. These parameters, together with the volume of distribution (V), were used later for the PK-PD modeling.

PD Modeling

The PD data was analyzed according to the indirect response and sigmoidal E_{max} modeling approaches.

The indirect response model no. I of Jusko (16,17) assumed that there is a constant input of the parasympathetic tone (HF peak) that is produced in a zero order kinetics (K_{in}) and removed in a first order kinetics with a rate constant K_{out} . The drug inhibits the production of the parasympathetic tone (inhibition of K_{in}) and thereby affects the magnitude of parasympathetic tone (i.e., the measured response) (Fig. 1). Drug concentration in the central compartment was fitted to the magnitude of response vs. time data using the following equations:

$$I_{HRV} = [I_{max} \cdot (C_p)^n] / [IC_{50}^n + (C_p)^n] \quad (3)$$

$$dR/dt = K_{in} \cdot (1 - I_{HRV}) - K_{out}R \quad (4)$$

where I_{max} is the maximal inhibition of K_{in} , C_p is the drug concentration at the central compartment ($C_p = X_p/V$), IC_{50} is the drug concentration that produces 50% of I_{max} , R is the magnitude of the measured response, and n is the shape factor. Sigmoidal E_{max} model was described by the following equation:

$$E = E_0 - [E_{max} \cdot (C_p)^n] / [EC_{50}^n + (C_p)^n] \quad (5)$$

where E is the magnitude of the measured response ($E = R$ in the indirect model), E_0 is the baseline response, E_{max} is the maximal inhibition, C_p is the drug concentration at the central compartment ($C_p = X_p/V$), EC_{50} is the drug concentration that produces 50% of E_{max} , and n is the shape factor.

The indirect and E_{max} PK-PD models were fitted to the experimental concentration-response-time data with ADAPT II Pharmacokinetic/Pharmacodynamic Systems Analysis Software (Biomedical Simulations Resource, Los Angeles, CA) applying the mean likelihood procedure. The PK-PD fitting procedure was conducted with fixed PK parameters and was

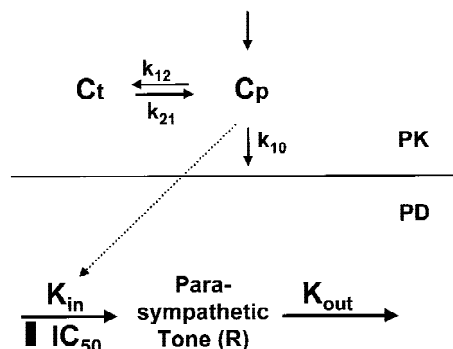


Fig. 1. The PK-PD model of atropine action on the parasympathetic tone according to the indirect response model. The PK is according to a two-compartment model, and the concentration in the central compartment (C_p) is linked to the inhibition of the production rate (K_{in}) of the parasympathetic tone. R is the magnitude of the measured response, K_{out} is elimination rate of the response, and IC_{50} is the drug concentration that produces 50% of maximal response (I_{max}).

applied for mean response-time data (0.5 mg/kg, $n = 4$; 1.0 mg/kg, $n = 10$; and 2.0 mg/kg, $n = 4$). Goodness of fit was determined according to Akaike (AIC) and Schwarz (SC) criteria (18).

RESULTS

PD Measurements

The suggested method enables the quantification of the parasympathetic inhibition in a graded manner and therefore allows one to follow the kinetics of atropine action. To demonstrate the technique, a cumulative plot of the HF component following administration of atropine (1.0 mg/kg) to rats is presented in Fig. 2. Refined stepwise regression divides the cumulative plot into short segments of data in which unified

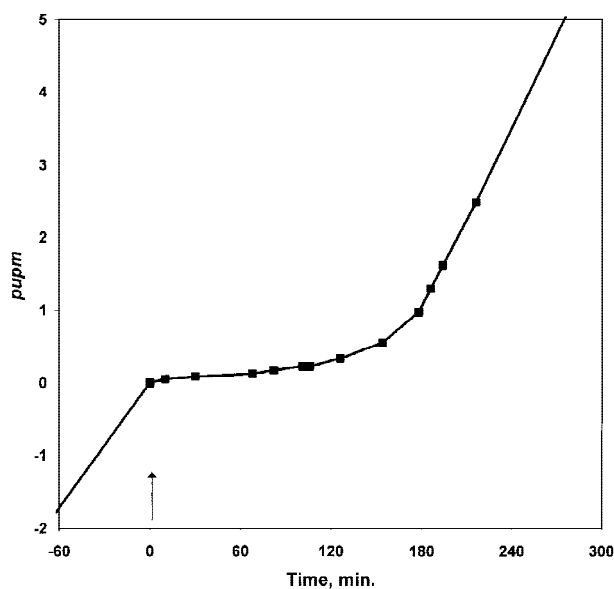


Fig. 2. Illustration of the temporal cumulative technique. The cumulative plot of the HF peaks following administration of atropine 1.0 mg/kg to rats is divided into short segments of data with consistent parasympathetic behavior. The parasympathetic tone at each segment is depicted by the slope of the cumulative plot (in units of pupm). Arrow indicates the time of drug administration.

Table I. Estimated PK and PD Parameters of Atropine

PK parameters				
Parameter	Estimate	% CV		
k_{10} (min^{-1})	0.043	7.20		
k_{12} (min^{-1})	0.154	21.8		
k_{21} (min^{-1})	0.082	24.2		
Volume (l/kg)	3.506	18.2		
PD parameters				
Parameter	Indirect model		Emax model	
	Estimate	%CV	Estimate	%CV
K_{in} (pupm/min)	0.78	116	—	—
I_{max}	0.80	1.2	0.15	11.2
IC_{50} (ng/ml)	15.2	3.4	15.5	3.4
n^a	5	—	5	—
Model selection criteria	AIC	-559	AIC	-197
	SC	-537	SC	-179

^a Fixed parameter.

parasympathetic behavior is found. The parasympathetic tone (R) is characterized for each segment by the slope (in units of pupm), and duration (min). A certain temporal delay exists between the concentration-time and response-time curves (see Fig. 3).

The response-time data following administration of three different doses of atropine is presented in Fig. 4. Maximal inhibition of parasympathetic tone (80% of the baseline level) was attained shortly after drug administration at all three doses, and continued for 50, 80, and 100 min after drug administration for the 0.5, 1.0, and 2.0 mg/kg doses, respectively, and was followed by gradual return to baseline level.

PK Analysis

A two-compartment open PK model appropriately described the concentration-time profile of atropine following bolus administration to rats with a rapid distribution phase ($t_{1/2}$ alpha = 3 min) followed by a slow elimination phase ($t_{1/2}$ beta = 43 min). The calculated PK parameters are summarized in Table I and the observed vs. predicted results are presented in Fig. 3.

PK/PD Modeling

The changes in parasympathetic tone following administration of atropine at three different doses are presented in Fig. 4 together with the simultaneous fit of the proposed indirect response model. The estimation of the PD parameters was based on fixed PK values as well as fixed value of the shape factor ($n = 5$) that was determined according to preliminary modeling steps. K_{out} was fixed as a function of K_{in} and the baseline response ($K_{out} = K_{in}/R_0$). The estimated PD parameters according to the sigmoidal E_{max} model and the indirect response model are summarized in Table I. The values of AIC and SC obtained for the indirect model were 3-fold lower than those obtained for the sigmoidal E_{max} model.

DISCUSSION

The time interval between heartbeats is not constant and the variability that occurs is due to a variety of physiological processes that affect the heart rate at different frequencies.

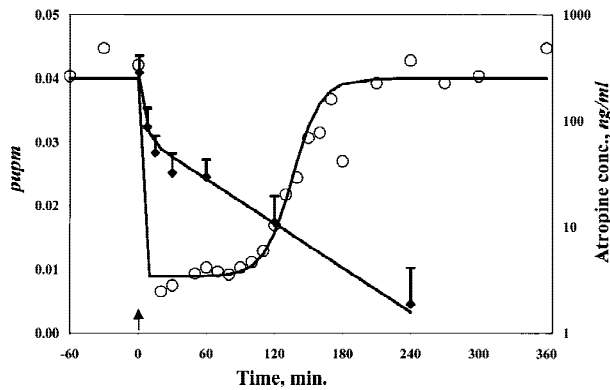


Fig. 3. Plasma atropine concentrations (\blacklozenge) and parasympathetic tone (pupm, \circ) following bolus intravenous administration of 1.0 mg/kg atropine. The data points are the observed data, and solid lines are the best fits according to the PK and the indirect response models.

The measured tachogram of HRV is a reflection of all the different interacting phenomena. PSA of HRV divides the entire variability into separate frequencies, and thereby enables the identification of the activity of the affecting components (4,19). This noninvasive technique has gained considerable interest in both cardiology (7,20) and neurology (21–23).

The HF component of PSA of HRV is considered a reliable indicator of parasympathetic efferent activity (19,24), and is regarded as an important measure of parasympathetic tone (4). However, thus far the PSA-HRV has mainly been used only as a qualitative method due to the profound “noisy” data that it yields.

Recently, we proposed a new approach to overcome the inherent non-stable (noisy) data that characterizes the ANS (10). This approach is based on analysis of the cumulative plot of the HF peaks obtained by PSA of HRV vs. time. This plot constructs a clear pattern of the autonomic tone and illustrates the impact of drug treatment on the parasympathetic system. In this work we take this approach one step further to apply it as a practical PD tool. By applying a retrospective statistical method on the cumulative approach, the temporal changes in the parasympathetic activity following drug admin-

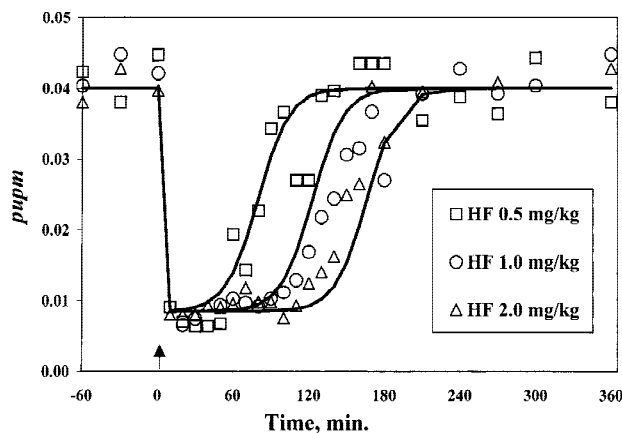


Fig. 4. The observed parasympathetic tone (HF) vs. time data following administration of 0.5 (\square), 1.0 (\circ), and 2.0 (\triangle) mg/kg atropine. Curves show the simultaneously fitted profiles according to the indirect PD model described in Fig. 1.

istration are identified in a quantitative manner. As the data obtained by PSA of HRV is non-stable, to investigate the kinetics of drug action, an averaged value of certain time epochs is required. These time epochs should be short when the changes in the autonomic tone are rapid, and greater when the changes are moderate. Therefore, we applied a new approach that determines the beginning and end of each “response step” by dividing the cumulative plot into smaller slopes that captures the curve (as shown in Fig. 2). Each slope represents a time interval with relatively stable parasympathetic tone. These time epoch are short when the magnitude of effect of the drug is rapidly changing, where as larger time epochs are used to describe moderate changes in parasympathetic tone. The slope of each epoch, (in units of pupm) serves as a quantitative PD measurement that is used for the PK-PD analysis. A drawback of the temporal cumulative approach is the fact that it cannot be used online, as it requires long-term data records to identify segments of stable parasympathetic tone.

To describe the kinetics of atropine parasympatholytic activity *in vivo* we linked the intensity of effect, measured by the temporal cumulative method, to the blood drug concentration-time data using different PK-PD modeling approaches. Based on the lower AIC and SC values we concluded that the indirect response model better described the PD data than the sigmoidal E_{max} model (see Table I). In view of the fluctuations in the observed data, we concluded that the indirect response model appropriately captured the changes in the magnitude of the autonomic tone in response to the drug effect, although certain deviations exist between the observed data and fitted model. The simultaneous fit of common parameters that describe the kinetics of atropine action for different doses contributes to the power of the estimated values as compared to the parameters that can be estimated based on each dose alone (18), and enables the prediction of the kinetics of action of atropine following administration at similar doses in a rat model.

The indirect PD response modeling approach that was selected to relate concentration effect-time data enables the description of clinical and pharmacological phenomena in a mechanistically based manner (25), and is especially useful in case of hysteresis in a concentration response-time plot. The basis of this approach is that the measured response is a physiological parameter (parasympathetic tone in this case), and that the drug has a direct effect on the rate of production (or elimination) of this parameter and thereby affects indirectly the magnitude of the measured response. According to the known pharmacology of atropine (26), the parasympatholytic activity reduces the HF peak of HRV by vagal-nerve blockade (6). In the PD model utilized here, this activity is translated to reduced production of parasympathetic tone, which is in accordance with model no. 1 of Jusko (16,17).

The value of IC_{50} (15 ng/ml) estimated by the indirect response model proposes that at times where serum atropine concentration exceeded the IC_{50} levels (at 1, 2, and 2.5 h for doses of 0.5, 1, and 2 mg/kg, respectively), the production of parasympathetic tone is strongly inhibited (up to a maximum of 80% of the baseline K_{in} value). There is a certain delay between the time when IC_{50} is reached and when 50% of parasympathetic inhibition was observed (as shown in Fig. 3). The high value of the shape factor that was required to achieve best fit of the model to the observed PK-PD data

indicates that the inhibitory effect is characterized with a steep PD profile, which is close to an all-or-none profile. This means that significant inhibition begins at a concentration close to the IC_{50} and maximal activity is already attained at concentrations that are somewhat above IC_{50} (27).

There are certain similarities between our findings in a rat model using the indirect PD response approach and the work of Scheinin *et al.* (28), who modeled the PK-PD of atropine parasympatholytic effect in humans using the "effect compartment" model. In both cases, there was a hysteresis in the concentration-effect data that did not allow a good fit of the data to a direct PD model (i.e., E_{max} model). Although the "effect compartment" approach attributes the hysteresis to a PK phenomenon of slow distribution of the drug to the site(s) of action, the indirect response model ascribes the delayed response to the inhibitory action of the drug on the production rate of the measured effect. In addition, although using a different modeling approach in both human and rat PD data analysis, the PD profile was characterized with a steep concentration effect profile. The comparison between the two investigations also highlights the complexity of analyzing HRV data in a rat model. In humans, part of the "noise" associated with HRV data acquisition can be reduced due to the collaboration between the investigator and the tested subject, and the fact that they voluntarily stay in a predetermined posture during HR measurements. Although the HRV data is more variable in rats, the animal model enables the carrying out of preclinical investigations with unapproved drugs and where safety issues are of concern; thus, it serves as a fundamental step in the new entity (drug) development process. This work is the first to utilize PSA of HRV in a preclinical investigation to evaluate the kinetics of action and that relates the measured effects to drug concentration. The proposed approach demonstrates that PK-PD analysis can be executed in a rat model despite the inherent noisy HRV data.

In conclusion, we have utilized the PSA of HRV data as a quantitative measure of temporal changes in parasympathetic tone. We have shown that it can be used to measure the kinetics of drug action on the ANS. The rat model that has been established here paves the way for further PK-PD preclinical investigations in this field.

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