ATENOLOL EXPOSURE AS A RISK FOR ADVERSE METABOLIC EFFECTS TO BETA BLOCKERS

By

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To my parents

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LIST OF ABBREVIATIONS

CVD	Cardiovascular disease
HTN	Hypertension
BB	Beta blockers
BP	Blood pressure
B1AR	Beta 1 adrenergic receptor
AME	Adverse metabolic effects
PK	Pharmacokinetics
HDL-C	High density lipoprotein-cholesterol
TC	Total cholesterol
ISTD	Internal standard
LC/MS/MS	Liquid chromatography/mass spectrometry/mass spectrometry
HILIC	Hydrophilic interaction chromatography
QC	Quality control
C_{max}	Peak concentration
T_{max}	Time to achieve peak concentration
Cp	Plasma concentration
$T_{1/2}$	Elimination half life
AUC	Area under curve
AUCn	Area under curve, normalized
OGTT	Oral glucose tolerance test
HOMA	Homeostatic model assessment
BMI	Body mass index

Abstract of Thesis Presented to the Graduate School
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Cardiovascular disease (CVD) is the leading cause of mortality in the United States of America (USA) and hypertension (HTN) is an important CVD risk factor. It is also a risk factor for stroke, heart failure and renal failure. Beta-blockers (BB), along with diuretics have been among the consensus guideline recommended first-line agents for the treatment of HTN in the USA. BBs are widely used for treating HTN for many decades for their efficacy. However, many clinicians are concerned about the use of BB as a primary therapy for HTN due to the risk for adverse metabolic effects (AME) on glucose, insulin and lipids thereby the development of diabetes and dyslipidemia. However, these effects are observed only in certain fraction of the population. This suggests that there are other factors that predispose certain individuals to develop these AME. In our project, we determined if atenolol plasma concentration (Cp) as a factor may contribute to the inter-individual differences. We hypothesized, increasing systemic exposure to atenolol leads to increased risk for AME from atenolol therapy in HTN. We performed a 24-hour pharmacokinetic (PK) study in 15 hypertensive patients taking chronic atenolol 100 mg daily. We also measured their glucose and insulin levels by performing 2 hour oral glucose tolerance test (OGTT) and also measured their fasting lipids, glucose and insulin before the start of atenolol treatment and after 8 weeks of treatment. We did not found any

association between atenolol Cp and glucose levels during the OGTT and between atenolol Cp and change in lipid levels during the study duration. We did not find atenolol Cp as a risk factor for the development of AME. However, our study has a small sample size and restricts our power. The information from this study may lead to future studies that will help us identify those people at risk of these AMEs from BB, so that we can prevent or avoid the AMEs. Ultimately, we can avoid or prevent the occurrence of AME, and these important anti-hypertensive agents can still be safely used rather than being rejected totally.

CHAPTER 1
INTRODUCTION

Hypertension (HTN) is the most common chronic disease, affecting approximately 1 billion people worldwide and approximately 72 million Americans, with an additional 25 million Americans considered to have prehypertensive blood pressure (BP) [120-139/80-89 mmHg] (Chobanian et al., 2003). Stroke, heart failure, ischemic heart disease (including acute myocardial infarction and angina), and chronic renal failure are adverse outcomes associated with HTN. The pathophysiology of HTN in the approximately 90% of patients with essential HTN is not completely understood. HTN has numerous potential causes, resulting from abnormalities in the kidneys, vasculature and/or neurohormonal systems. Epidemiologic evidence demonstrates that the risks of cardiovascular morbidity and mortality rise progressively with increasing blood pressure (Lloyd-Jones et al., 2009).

Diabetes is also one of the leading causes of morbidity and mortality in the United States of America (USA). Type 2 diabetes comprises approximately 90% of new cases of diabetes and is a common concomitant disease with HTN (Reaven et al., 1996). Specifically, HTN occurs in 24% of the adult population in the USA, but in up to 60% of type 2 diabetics aged 45–75 years (Jacob et al., 1999). Notably, the risk of developing new onset diabetes is related to the antihypertensive treatment regimen (Elliott and Meyer, 2007).

Beta adrenergic receptor blockers (BB) have been used to treat high BP for more than four decades and have proven to reduce morbidity and mortality. BBs along with diuretics have been recommended as first line drugs for treating HTN for several decades. However, results from various large clinical trials indicate that patients taking thiazide diuretics and BB have a higher risk of developing type 2 diabetes (Hansson et al., 1999; Brown et al., 2000; Gress et al., 2000; Lindholm et al., 2003; Nilsson and Berglund, 2006; Stump et al., 2006). These drugs

decrease insulin sensitivity thereby causing type 2 diabetes (Pollare et al., 1989b; Reaven et al., 1996; Bonner et al., 1997; Giugliano et al., 1997; Reneland et al., 2000; Poirier et al., 2001; Sarafidis and Bakris, 2006). BB and thiazide diuretics are also associated with adverse lipid effects particularly on triglycerides, high density lipoprotein cholesterol (HDL-C) and total cholesterol (TC) (Day et al., 1979; Eliasson et al., 1981; Day et al., 1982; Weiner and Rossner, 1983; Otterstad et al., 1992; Rabkin et al., 1994). Recently, the effectiveness of BB based treatment for HTN has been questioned and many clinicians have argued against the use of BB as a first line therapy in HTN (Beevers, 1998; Messerli et al., 2003; Carlberg et al., 2004; Pepine and Cooper-Dehoff, 2004; Williams, 2007). Recent meta-analysis have found that BB increased the risk for new-onset diabetes with no benefit on the end point of death or myocardial infarction and increased risk for stroke by 15% compared with other agents (Lindholm et al., 2005; Bangalore et al., 2007b). Evidence from several clinical outcome trials demonstrate that compared to other anti-hypertensive classes, BB were no more effective at preventing cardiovascular events, resulting in BB being moved to fourth line treatment option for HTN in the latest UK and European guidelines (Chobanian et al., 2003; Mayor, 2006; Sever, 2006; Mancia et al., 2007). However, BB still hold a key role in treating patients with hypertension and other concomitant CVD, and remain a first line option in JNC 7 guidelines along with other drugs (Kendall, 1998; Cruickshank, 2000; Messerli et al., 2003; Fardoun, 2006; Nilsson and Berglund, 2006; Bangalore et al., 2007a; Cruickshank, 2007; Roberts et al., 2007). Given that the occurrence of adverse metabolic effects (AME) from BB, particularly diabetes, may offset their clinical benefit, it is important to identify the factors that place a patient at risk for these adverse effects, such that BB therapy might be avoided in those patients who are at risk.

Different BBs have variable effects on glucose, insulin and lipid levels. Nonselective BBs like propranolol have the most significant effects in decreasing insulin sensitivity, HDL-C and increasing triglyceride levels. Selective BB which specifically bind to the beta 1 adrenergic receptor (B1AR) have intermediate effects in reducing insulin sensitivity and HDL-C and increasing triglyceride levels. These drugs are prone to lose specificity at higher dose. Newer BBs like carvedilol, which have vasodilating properties, are found to improve insulin sensitivity and have a neutral effect on lipids(Jacob et al., 1998). (Table 1-1)

The mechanisms by which BB treatment modifies insulin sensitivity and affect lipids and cardiovascular risk factors are not fully understood. Insulin secretion and insulin clearance is reduced by BB usage (Pollare et al., 1989a; Pollare et al., 1989b; Jacob et al., 1998). BB also have significant adverse effects on triglycerides and HDL-C, which may result from their action in decreasing Lipoprotein lipase (LPL) and Lecithin cholesterol acyl transferase (LCAT) activity and altering cholesterol synthesis (Grimm, 1991; Rabkin et al., 1994; Liggett et al., 2006).

BB Usage and Occurrence of AME

The dose/concentration relationship between AME and thiazide diuretics is well established, but this is not the case for BB (Black, 1996; Savage et al., 1998). Low dose diuretics are found to have neutral effects on the development of new onset diabetes. There are many conflicting studies that have compared the effects of BB on lipid levels and insulin sensitivity. Some studies have found an association between short term use of BB and hypertriglyceridimia while others have not. Table 1-2 summarizes some of the studies which show the conflicting reports about BB effect on lipid levels, while Table 1-3 summarizes some of the studies about BB effect on insulin sensitivity.

Atenolol

Atenolol is a polar hydrophilic phenylacetamide [(4-2'-hydroxy-3'-isopropyl-aminopropoxy) phenylacetamide] (Figure 1-1). It is a selective B1AR antagonist devoid of any intrinsic sympathomimetic and membrane stabilizing activity (Barrett et al., 1973; Mason et al., 1979). In humans, there is incomplete absorption after oral administration (Brown et al., 1976; Fitzgerald et al., 1978; Tabacova and Kimmel, 2002). Hence, the systemic bioavailability is approximately 50%. Peak plasma levels after oral administration are achieved after 2-4 hours. The absorbed drug is widely distributed in the body(Reeves et al., 1978b), but only a fraction of the administered dose reaches the brain. It is minimally bound to plasma proteins, <5%, about 10% of the drug is metabolized with the rest eliminated unchanged in urine by filtration (Reeves et al., 1978a; Reeves et al., 1978b). After intravenous administration, complete drug is eliminated in the urine whereas upon oral dosing, 40-50% of the unchanged drug is recovered in urine and the rest is excreted through feces due to incomplete absorption (Tabacova and Kimmel, 2002). The elimination half life $(t_{1/2})_{is}$ between 6-8 hours. As atenolol is cleared by glomerular filtration, the elimination half life is prolonged in patients with renal disease.

There is significant variability reported in atenolol pharmacokinetic parameters. There is a fourfold variation in the plasma concentrations, three fold in $t_{1/2}$ and peak plasma concentrations (Cmax), and two fold in time to achieve peak plasma concentration (T_{max}) (Brown et al., 1976; Fitzgerald et al., 1978; Mason et al., 1979; Melander et al., 1979; Barnwell et al., 1993; Shiga et al., 1993; Sowinski et al., 1995; Sourgens et al., 2003). Some of the factors associated with this variability are age, disease status and presence of food. Young men had lower plasma concentrations and higher apparent oral clearance CL/F than older men which can be due to age related decline in renal function, and changes in body composition (Greenblatt et al., 1982; Sowinski et al., 1995). Similarly, as renal function declines, the clearance decreases

and the elimination half-life increases significantly (Sassard et al., 1977; McAinsh et al., 1980). Presence of food has also been shown to affect the absorption of atenolol. Food intake decreased the plasma concentration by 20% (Melander et al., 1979), co-administration of atenolol with orange juice also significantly decreased the plasma concentration by 40% (Lilja et al., 2005). The reasons behind the food associated reduction in bioavailability are believed to be the release of bile acids after food intake and the complexation of the hydrophilic drug in the bile acid micelles (Barnwell et al., 1993). Atenolol pharmacokinetic variability might affect the development of the metabolic adverse effects on insulin sensitivity and lipids.

The Scope of Present Work

Despite the body of literature documenting the AME of BB, there are no studies that have addressed the relationship between the atenolol plasma concentration and the occurrence of the drug induced change in triglycerides or change in insulin sensitivity. Also, as mentioned above there is significant variability in the pharmacokinetic parameters of atenolol which can potentially affect the development of the AME in different individuals. We hypothesize that the occurrence of the metabolic abnormalities with atenolol are concentration related and that people with higher atenolol area under the plasma concentration-time curve (AUC) are those who are more likely to develop these AME. For testing this hypothesis, we conducted a 24 hour pharmacokinetic (PK) study in hypertensive patients who were dose titrated to100mg atenolol for 4 weeks after taking atenolol 50mg for 3-4 weeks. We also measured their glucose/insulin area under the curve by doing a 2 hour glucose tolerance test and their change in lipid levels from the time they started taking drug to the PK study visit.

Table 1-1. Effects of BB treatment on metabolic risk factors

Compound	Insulin sensitivity	Triglycerides	HDL-C	TC
Propranolol	-33%	+25%	-10%	+9%
Metoprolol	-21%	+30%	-7%	-1%
Atenolol	-22%	+18%	-9%	=
Pindolol	-17%	=	=	=
Dilevalol	+10%	-22%	=	-6%
Carvedilol	+13%	=	=	=
Celiprolol	+35%	-15%	+5%	=

- Decreased, + increased, = no change (Adapted from Jacob et al. 1998)

Table 1-2. Studies assessing the effects of atenolol and other BB on lipid profile

Author	Study duration	n	Results
Day et al. 1979	6-8 months	49	Atenolol (50mg-100mg bid) had 24% increase, propranolol (40-160mg tid) had 60% increase in basal triglyceride levels
Eliasson et al, 1981	15 months	15	Atenolol 100mg increased triglyceride concentrations by 30%
Day et al. 1982	12 months	53	Increase in triglycerides were observed as follows: atenolol 100mg (24%), propranolol 160mg (51%), metoprolol 200mg (14%), oxeprenolol 160mg (26%)
Weiner and Rossner, 1983	3 months	50	Atenolol 50 mg once daily did not change the serum lipoprotein levels while metoprolol 200mg increased the lipoproteins
Otterstad et al., 1992	3-12 months	100	Atenolol 50mg once daily increased triglycerides by 15% and decreased HDL by 5% compared with hydrochlorthiazide (HCTZ) 25mg–amiloride 5mg combination
Rabkin et al, 1994	6 months	131	Atenolol increased triglycerides by 18%, decreased HDL by 7% as compared to doxazosin

Table 1-3. Studies assessing the effects of atenolol and other BB on glucose and insulin

Author	Study duration	n	Results
Pollare et al., 1989b	16 weeks	60	Glucose uptake decreased for both atenolol and metoprolol causing increase in fasting plasma insulin, blood glucose, hemoglobin A1c
Bonner et al., 1997	12 weeks	32	Glucose AUC in the IV glucose tolerance test increased during atenolol therapy
Giugliano et al., 1997	24 weeks	45	Fasting plasma glucose concentrations and insulin levels increased with atenolol while they decreased with carvedilol
Reneland et al. 2000	48 weeks	26	Insulin sensitivity decreased 23% by atenolol
Poirier et al., 2001	16 weeks	25	Atenolol was associated with 20% greater reduction in insulin sensitivity than with nebivolol

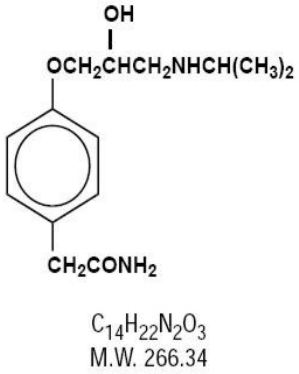


Figure 1-1. Atenolol chemical structure

CHAPTER 2
ATENOLOL LC/MS/MS ASSAY

Introduction

Atenolol is a selective B1AR antagonist that is commonly used to treat hypertension (Barrett et al., 1973; Mason et al., 1979). Numerous methods are reported in the literature for the determination of atenolol concentration in biological fluids including gas chromatography with mass spectrometry (GC-MS), high performance liquid chromatography (HPLC) or capillary zone electrophoresis (Abdel-Hamid, 2000; Li et al., 2005). Although GC-MS is a sensitive technique, the sample preparation requires derivatization, which is tedious and time consuming. HPLC particularly reversed-phase liquid chromatography is widely used for analysis of atenolol with mobile phases consisting of acetonitrile or methanol, buffer, an ion pairing reagent (e.g. alkyl sulphonates) to provide adequate retention and organic amines (e.g. triethyl amine) to reduce peak tailing (Verghese et al., 1983). But these additives can shorten life of reversed-phase packings such as C_{18} silica (Basci et al., 1998). Moreover, atenolol is a polar compound and hence it is difficult to retain on a C_{18} column, except when using a highly aqueous mobile phase, which can collapse high density C_8 or C_{18} columns (Naidong, 2003). Basic compounds can be separated on plain, unbonded silica with aqueous-rich mobile phases or with organic-rich eluents, employing hydrophilic interaction chromatography (HILIC), (Jeong et al., 2007; Hsieh, 2008). In HILIC, analytes elute by passing a hydrophobic or mostly organic mobile phase across a neutral hydrophilic stationary phase causing solutes to elute in order of increasing hydrophilicity resulting in better separation of highly polar compounds(Li et al., 2005). We adapted a HILIC method reported by Li et al(Li et al., 2005) to analyze atenolol in human plasma.

Experimental

Chemicals and Reagents

Atenolol reference standard and atenolol-d7 were obtained from MP Biomedicals LLC, (Solon, OH, USA) and Toronto Research Chemicals, (North York, ON, Canada) respectively. Acetic acid and trifluoro acetic acid were purchased from VWR, (West Chester, PA, USA). Acetonitrile was obtained from Fisher Scientific, (Waltham, MA, USA). Blank plasma was obtained from Shands hospital (Gainesville, FL, USA). HPLC grade deionized water was obtained from a Barnstead Nanopure Diamond UV Ultrapure Water system (Dubuque, IA, USA).

Preparations of Standards and Quality Control (QC) samples

A stock solution of atenolol was prepared at a concentration of 1mg/mL in acetonitrile and stored in a glass vial at 4^0C. Dilutions prepared in acetonitrile at concentrations of 100, 10 and 1 µg/mL were used to prepare calibration standards and quality control (QC) samples. Blank plasma was spiked with appropriate stock solutions. Eight calibration standards were prepared at concentrations of 5,10,25,50,100,250,500 and 1000 ng/mL. QC samples were prepared at concentrations of 15 ng/mL (low QC), 200ng/mL (medium QC) and 750ng/mL (high QC). A dilution QC was prepared at a concentration of 2000 ng/mL. Standards and QC samples were prepared at the beginning of the validation, transferred into 1.5mL micro centrifuge tubes (Thermo Fisher Scientific, Waltham, MA, USA) and stored at -20^0C until analysis. A stock solution (1mg/mL) of the atenolol-d7, internal standard (ISTD) was prepared in acetonitrile and further diluted to 200 ng/mL and used for all analyses. The ISTD stock solutions were stored in glass vials at 4^0C.

Sample Preparation

Frozen samples were thawed at room temperature. An aliquot (100µl) of plasma was transferred to a 1.5mL micro centrifuge tubes (Thermo Fisher Scientific, Waltham, MA, USA). The plasma was combined with 50µl of ISTD (except for blank without ISTD), vortex mixed and 1mL acetonitrile was added, except for blank without ISTD and vortex for 1 minute. The tubes were centrifuged for 10 minutes at 14000 rpm in an Eppendorf 5810R centrifuge. The supernatant (800 µl) was transferred in a clean glass culture tube and briefly vortex-mixed. Then they were evaporated under nitrogen and reconstituted with 200µl acetonitrile. Samples were transferred to auto sampler vials with micro inserts and then injected into the system (20 µl). The auto sampler temperature was maintained at 10^{o}C.

LC/MS/MS Conditions

The LC/MS/MS system consisted of a ThermoFinnigan Surveyor HPLC auto sampler, ThermoFinnigan Surveyor MS quaternary pump and a ThermoFinnigan TSQ Quantum Discovery triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA, USA). The TSQ Quantum mass spectrometer was equipped with an electrospray (ESI) ion source and operated in the positive mode. The ESI source spray was set orthogonal to the ion transfer capillary tube. The mass spectrometer was calibrated with a solution of polytyrosine-1, 3, 6 according to the manufacturer. The MS/MS conditions were optimized by infusing atenolol in the mobile phase. The ESI source parameters were tuned for maximum abundance of $[M-H]^-$ ions of atenolol at the LC flow-rate of 0.22 mL/min. For quantification, the TSQ Quantum was operated in high resolution multiple reaction monitoring mode. The acquisition parameters were: spray voltage 3.5V, source CID 10 V, and heated capillary temperature at 350^{o}C. Nitrogen was used as the sheath and auxiliary gas and set to 35 and 15 (arbitrary units), respectively. The

argon collision gas pressure was set to 1.5 mTorr. The collision energy was 33 eV for atenolol and atenolol-d7. The selected reaction monitoring scheme followed transitions of the $[M+H]^+$ precursor to selected product ions with the following values: m/z 267.12 $\rightarrow$ 144.50 for atenolol and 274.20 $\rightarrow$ 144.50 for atenolol-d7. The instrument was operated in enhanced (high) resolution with peak width (FWHM) set to 0.2 m/z at Q1 and to 0.7 m/z at Q3. The scan time was 200 ms for each transition. MRM data were acquired and processed using ThermoFinnigan XCalibur® software version 1.4, service release 1 (Thermo Electron Corporation, San Jose, CA, USA).

Chromatographic Conditions

Chromatographic separation was performed using a Betasil Silica-100, 50 x 3 mm, 5 µ analytical column (Thermo-Electron Corporation, Bellfonte, PA, USA). The mobile phase used for the analysis was 94% acetonitrile, 6% deionized water, 0.5% acetic acid and 0.04% trifluoro acetic acid (TFA). The mobile phase was degassed and filtered through a 0.22 nylon membrane before use. The flow rate was 0.5 mL/min.

Standard Curve

Duplicate standard curves were analyzed in each run. For each standard calibration curve, the atenolol peak area to ISTD peak area ratio was calculated and plotted against nominal atenolol concentrations. Weighted ($1/concentration^2$) linear regression analysis was used to construct calibration curves from the standards. The regression equation was used to calculate the concentrations in quality control (QC) and clinical samples.

Method Validation

The current LC-MS/MS method was validated for precision, accuracy, linearity, dilution integrity, selectivity, carry-over, matrix effect, recovery and stability. The accuracy and precision of the assay was determined by the analysis of atenolol QC samples at concentrations of 15.0,

200.0 and 750.0 ng/mL. Six of each QC levels were analyzed daily for two runs and twelve of each QC levels were analyzed for one run. The difference in the calculated mean concentration relative to the spiked concentration was used to express accuracy (% deviation). Means, standard deviations and coefficients of variation were calculated from the QC values and used to estimate the intra- and inter-run precision. A dilution QC was prepared at a concentration of 2000 ng/mL. Six replicates of the dilution QC were processed after being diluted 10-fold.

Selectivity was evaluated by processing and analyzing blank plasma obtained from six sources. Carry-over was evaluated by placing vials of blank mobile phase at several locations in the analysis set. The potential for a matrix effect (suppression or enhancement of ionization) was evaluated by continuous infusion of atenolol post-column and injecting processed plasma samples from six different sources. There was no evidence of a matrix effect in the region where atenolol and the deuterated internal standard elute.

Extraction efficiency was determined by comparing the response obtained in the following samples: (A) quality control samples processed normally and (B) plasma samples extracted by the usual process and then spiked to contain the analyte and internal standard at concentration values corresponding to the QC concentrations (reference samples). This is done to eliminate any potential matrix effect. Responses obtained from reference samples were defined as 100%.

Processing and analysis of QC samples test the stability of the analytes (post-processing). The stability of the extracts in the auto sampler was evaluated after the extracts were left in the auto sampler at 10^{o}C for at least 40 hours. The analytes were found to be stable under these conditions. The effect of freeze and thaw on the stability of the samples was tested using low and high concentration QC samples. Three aliquot tubes of each QC were stored at 4^{0}C as a reference

and the other tubes were subjected to three freeze-thaw cycles prior to processing and analysis. The thawed samples were processed and analyzed as described above. Effects of freeze and thaw were measured by concentrations of each QC sample relative to the reference.

Application to Plasma Sampling

The method was used for the analysis of plasma samples obtained from hypertensive patients who were on atenolol 100mg for 4 weeks. The protocol was approved by the University of Florida Institutional Review Board and all study subjects provided written informed consent. Atenolol 100mg (Sandoz Inc., Princeton, NJ, USA) was given orally at the General Research Center at the University of Florida after 4 weeks of pretreatment with atenolol 100mg once daily. Blood samples were collected in tubes containing sodium heparin at multiple time points within the dosing interval; plasma obtained by centrifugation was stored at -20^0 C until analyzed. Plasma concentrations of atenolol were determined as described above. A non-compartmental model was used to describe atenolol pharmacokinetics. The maximum atenolol concentration (Cmax), time at which Cmax occurred (Tmax), apparent oral clearance (Cl/F, where F is bioavailability) and half life are reported. The pharmacokinetic parameter estimates were calculated using WinNonlin software (version 2.1, Pharsight Corporation, Mountain View, CA, USA).

Results and Discussion

Chromatographic Method

Representative LC/MS/MS chromatograms of plasma samples are shown in Fig. 2-1. Fig. 2-1A shows a double blank plasma sample (no atenolol or ISTD), Fig. 2-1B is a plasma sample spiked with atenolol-d7 (ISTD; 50 ng/mL), Fig 2-1C is lower limit of quantification (LLOQ) of atenolol, Fig. 2-1D depicts a plasma sample obtained 2.5 h after the dose of atenolol 100mg.

Retention time was approximately 1.6 min for atenolol. The peak of interest was well separated and free from interference with endogenous substances.

Method Validation

Linearity, Precision, Accuracy and Dilution Integrity

Linear calibration curves (n = 6) with a mean correlation coefficient of 0.9872 were obtained for atenolol over the concentration range of 5–1000 ng/mL.

The accuracy and precision data from QC samples demonstrate suitability of the method (Table 2-1). Intra- and inter-run precision (% CV) was ≤7% and accuracy (% deviation) was within ±13.3% (Table 2-1). The dilution QC (2,000 ng/mL) was processed after a 10-fold dilution with blank plasma to determine dilution integrity. The mean concentration found for the dilution QC was 2726 ng/mL. The mean precision (%CV) was 6.3% and the accuracy (% deviation) was 3.2%, which were within the acceptance criteria (<15%).

Selectivity, Matrix Effect, Recovery, and Stability

No endogenous interference with atenolol was detected in six different sources of blank plasma and there was no evidence of sample carry-over. The post-column infusion experiments used to assess a matrix effect showed no evidence of a change in signal in the regions where atenolol eluted. Further, the matrix effect assessed by spiking samples post-processing showed <10% difference from spiked injection solvent. The mean extraction recoveries for atenolol were 102% and 119% at concentrations of 750 and 15 ng/mL, respectively. Atenolol was stable in processed samples held in the auto sampler at 10 ◦C for at least 24 h with mean recoveries within 10%of the nominal concentration. No degradation of atenolol was observed in the samples subjected to three freeze–thaw cycles.

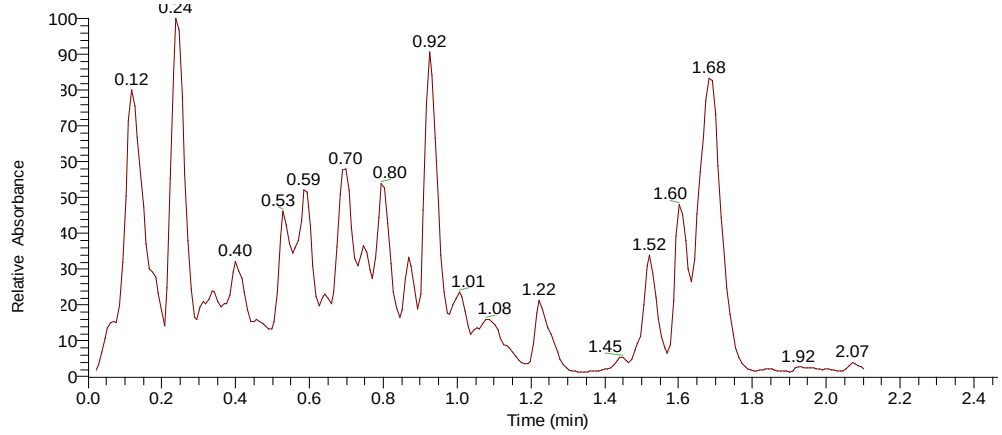
Method Application

The mean atenolol concentration–time profile obtained from 15 hypertensive patients after an oral dose of atenolol 100mg is shown in Fig. 2-2. The observed Cmax concentration of 1156±198 ng/mL occurred at 2.8±0.9 h. The apparent oral clearance of atenolol after oral drug administration (CL/F) was 176±54 mL/min and the estimated half-life was 6.9±1.7 h. The concentration of atenolol in all of the samples was greater than the lower limit of quantification, which was set at 5ng/mL. The results demonstrate that the assay is suitable for pharmacokinetic studies of atenolol in human subjects.

Conclusions

We have validated a LC–MS/MS method developed by (Li et al., 2005) for atenolol using protein precipitation. The method requires only a small amount of plasma (100μL) and can be applied to quantitate concentrations of atenolol in human plasma samples. The method was shown to be rapid, sensitive, selective, and reproducible. The method was used to determine atenolol concentrations in the pharmacokinetic study reported in Chapter 3.

A)



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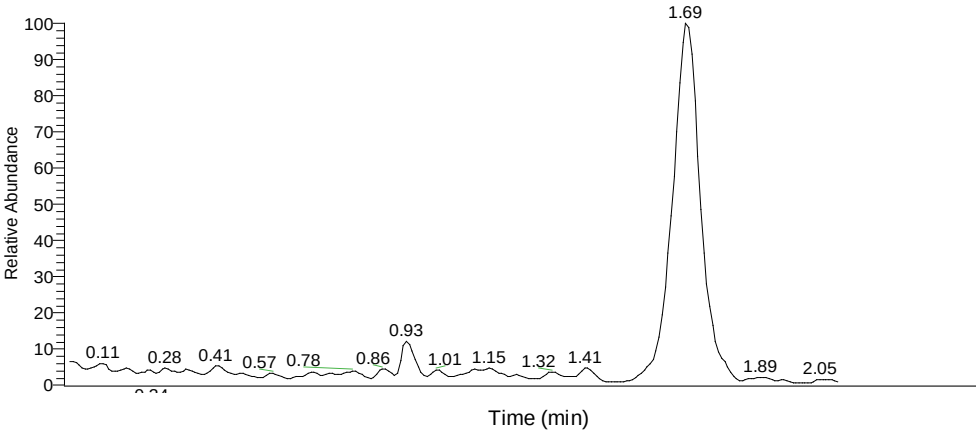
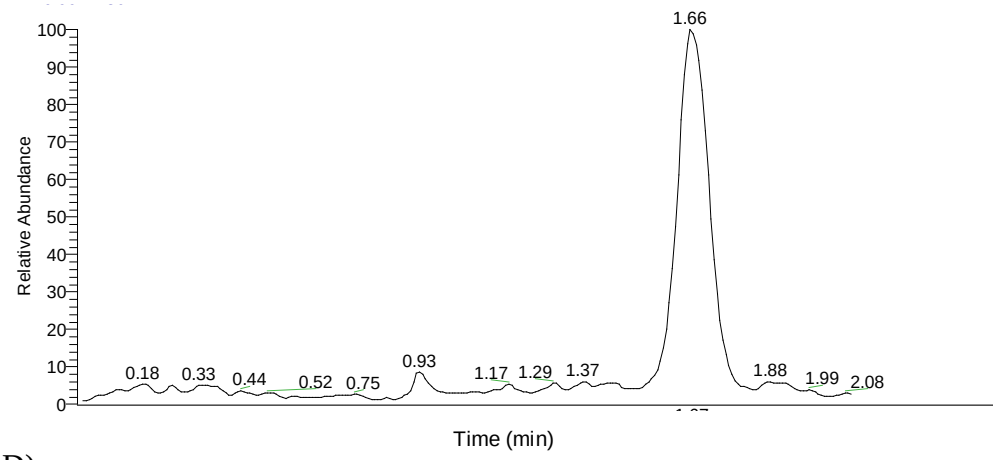


Figure 2-1. The extracted LC-MS/MS chromatograms of: A) double blank plasma (no atenolol or ISTD); B) Blank plasma with ISTD; C) atenolol lowest standard (5ng/mL); C) plasma sample from a patient obtained after 2.5hours after oral administration of atenolol 100mg and Blank with ISTD

C)



D)

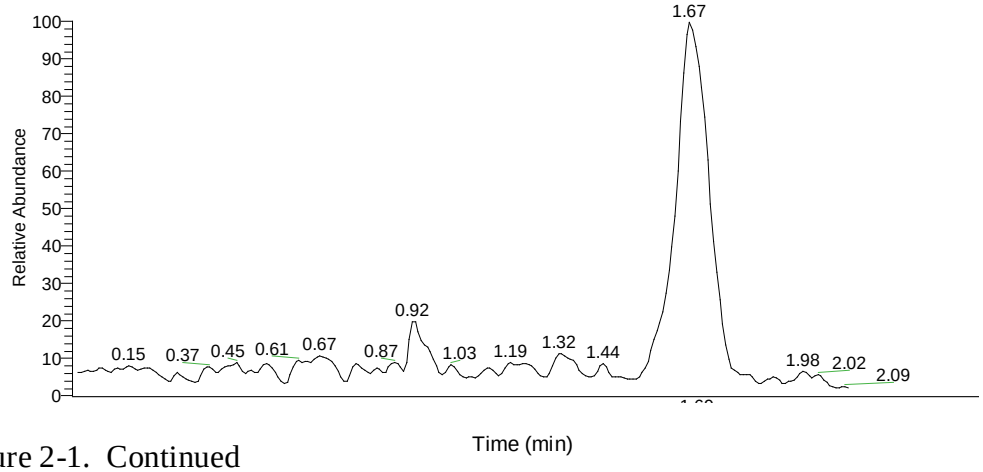


Figure 2-1. Continued

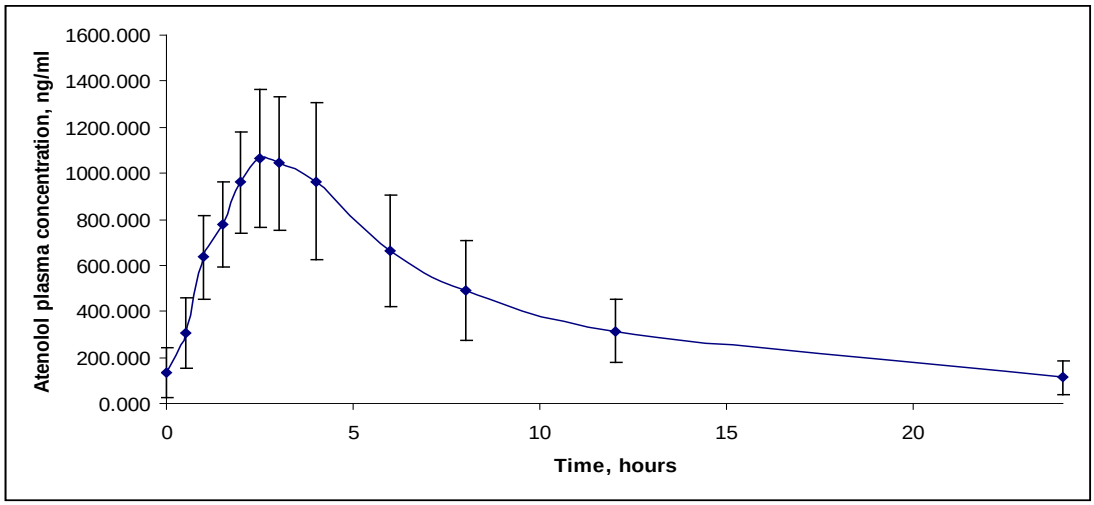


Figure 2-2. Mean (±SD) concentration time profile of atenolol after atenolol 100mg in fifteen patients

Table 2-1. Intra- and inter-run precision (%RSD) and accuracy (%RE) for atenolol quality control samples in human plasma

Concentration (ng/mL)		Precision (% CV)	Accuracy (%Deviation)
Nominal	Observed (mean± SD)		
	Intra-run (n=12)		
15.00	16.99±1.23	7.3	13.3
200.0	182.6±10.1	5.5	-8.7
750.0	733±51.5	4.6	-3.4
	Inter-run (n=24)		
15.00	16.78±11.9	7.8	11.9
200.0	190.05±10.91	5.7	-5.0
750.0	733±51.53	7.0	-2.3

CHAPTER 3
ATENOLOL PHARMACOKINETIC STUDY

Introduction

Atenolol is a selective B1AR blocker widely used for the treatment of HTN (Barrett et al., 1973; Mason et al., 1979). Despite the body of literature documenting the adverse metabolic effects (AME) of atenolol on glucose, insulin and lipids, there are no studies that have addressed the relationship between the atenolol plasma concentration and the occurrence of the drug induced change in triglycerides or change in insulin sensitivity (Day et al., 1979; Weiner and Rossner, 1983; Pollare et al., 1989b; Reaven et al., 1996; Bonner et al., 1997). Also, as mentioned earlier there is significant variability in the pharmacokinetic parameters of atenolol that can potentially affect the development of the AME in different individuals (Brown et al., 1976; Fitzgerald et al., 1978; Barnwell et al., 1993). The euglycemic hyperinsulinemic clamp is considered the gold standard for assessing insulin sensitivity (Uwaifo et al., 2002). However, it is a labor intensive, invasive and expensive method (Trout et al., 2007). Besides measuring simple fasting plasma glucose values, the oral glucose tolerance test (OGTT) is the most common method used for the diagnosis of type 2 diabetes by clinicians; it is cost effective and easy to perform, unlike the clamp method (American Diabetes, 2008). We hypothesize that the occurrence of the metabolic abnormalities with atenolol are concentration related and that people with higher plasma atenolol exposure are more likely to develop AME. For testing this hypothesis, we conducted a 24 hour pharmacokinetic (PK) study in hypertensive patients who were dose titrated to 100 mg atenolol for 4 weeks after taking atenolol 50 mg for 3-4 weeks. We also measured glucose and insulin plasma concentrations after a 2 hour OGTT and the change in lipid levels from the start of therapy until the time of the PK study visit.

Materials and Methods

Study Design

This was an open label study in hypertensive patients aged 18 to 65 years of any race, ethnicity and gender. Subjects were recruited from Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study. The details of PEAR study are as follows:

PEAR Protocol

PEAR is an ongoing hypertension pharmacogenetics study. It is a prospective, open label, randomized study of the beta blocker, atenolol and diuretic, HCTZ given as monotherapy or combination to determine which genes are associated with either antihypertensive response or AME (Johnson et al., 2009). Patients are recruited from the University of Florida (Gainesville, FL), Mayo Clinic (Rochester, MN) and Emory University (Atlanta, GA). These centers will recruit 800 mild to moderate essential hypertensive patients, male or female, of any ethnicity and between the ages of 17 and 65. Patients, who have secondary forms of HTN, isolated systolic HTN, heart rate < 55 beats/min, known cardiovascular disease, diabetes mellitus (Type 1 or 2), renal insufficiency, primary renal disease, pregnancy or lactation are excluded from the PEAR study.

Subjects who are enrolled in PEAR and randomized to atenolol as their first study drug were asked by the PEAR study personnel about their interest in learning more about the PK study and if they expressed interest, they were contacted to have the study explained to them. Subjects from PEAR who are on atenolol 100 mg once daily were invited to participate. The protocol was approved by the University of Florida Institutional Review Board and all study subjects provided written informed consent.

Inclusion criteria: We enrolled all patients at the University of Florida who were randomized to atenolol monotherapy in the PEAR study, were on 100mg dose and consented to participate.

PK Study protocol: Participants came to the University of Florida, General Clinical Research Center (GCRC) as an outpatient after four weeks on 100 mg atenolol. They were asked to avoid any fruit juices for four days before the study visit. Patients fasted for 8hours before reporting to the GCRC at 8am on the study day. After a basic clinical examination including height, weight, blood pressure and heart rate; a forearm vein of the subject was cannulated with a plastic catheter. Fasting lab measurements of glucose, insulin, triglycerides, HDL-cholesterol, total cholesterol were done. At 8.30am they were given an atenolol 100mg tablet (Sandoz Inc., Princeton, NJ, USA). Blood was drawn at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 hours after atenolol dosing into heparinized vacutainers for analysis of atenolol. Blood samples for measuring HDL-cholesterol, triglycerides and total cholesterol were collected in vacutainers with EDTA (Becton-Dickinson, Franklin Lakes, NJ, USA).

All patients underwent a two hour OGTT, one hour after atenolol dosing, whereby they were given 75g glucose solution (S/P lemon lime glucose tolerance beverage, Cardinal Health, Mcgaw Park, IL, USA) to drink. Glucose and insulin were measured during the OGTT at every 30 minutes by drawing blood in vacutainers with sodium fluoride for the measurement of glucose and in serum separator tubes for insulin. Following separation of the plasma, the samples were frozen and stored at -20 ^{0}C until assayed. Regular meals were served after the end of OGTT. Patients went home after the 12 hour blood sample and returned for the final 24 hour sample.

Laboratory Measurements

Atenolol was assayed by LC/MS/MS using a validated method as described in Chapter 2. Glucose was measured on YSI model 2300 STAT PLUS analyzer using glucose oxidase enzyme in the GCRC, University of Florida. Insulin was measured with Roche/Hitachi Modular® by

Shands laboratories, Gainesville, FL, USA. Lipids were measured using spectrophotometric methods by Quest diagnostics, Tampa, FL USA.

Pharmacokinetic Data Analysis

The measured atenolol Cp was evaluated using non-compartmental pharmacokinetic data analysis using WinNonlin software (version 2.1, Pharsight Corporation, Mountain View, CA, USA). Terminal elimination half-life ($t_{1/2}$) was assessed by non-linear regression. AUC within one dosing interval (AUC_{0-24}) at steady state was calculated using the trapezoidal rule. Maximum concentration C_{max} and time of maximum concentration T_{max} was observed directly from the measured data. The concentration time profile of the fifteen patients is shown in Figure 3.1.

Pharmacodynamic Data Analysis

Homeostatic model assessment (HOMA) of insulin resistance, HOMA-IR was calculated as the product of basal glucose (mmol/L) and insulin (μIU/mL) divided by 22.5 and HOMA of β-cell function (HOMA-BC) computed as product of 20 and basal insulin (μIU/mL) divided by value of basal glucose (mmol/L) concentration minus 3.5. The AUC for glucose and insulin during the OGTT (AUC_{0-2}) were calculated by the trapezoidal rule.

Statistical Methods

With a sample size of 15 patients, we have 80% power at P=0.05 two-sided to detect a target population correlation of 0.62 between log atenolol AUC and log glucose AUC. The use of natural logarithms limits the effects of potential outliers. We normalized atenolol AUC by body weight prior to log transformation. Descriptive statistics are given as means ± SD. We also fitted a multiple regression model of log atenolol AUC against log glucose AUC, log insulin AUC and change (Δ) in triglycerides. As a secondary analysis, we looked at the association with log atenolol AUC with (Δ) in glucose, (Δ) insulin, (Δ) HOMA, (Δ) HDL, (Δ) total cholesterol

with stepwise adding weight, BMI, waist circumference and hip circumference in the model. The change from baseline lab values were compared with paired t-test. All statistical analyses were conducted using SAS software (version 9.1, SAS Institute, Cary, NC, USA).

Results

A total of twenty patients were enrolled and fifteen patients completed the study (6 men, 9 women). Four patients declined to participate after consenting and one patient was consented while taking the 50 mg dose but was never titrated to the 100 mg dose. The patient characteristics are reported in Table 3-1. The mean age was 46±8.9 years, the majority of patients were women (60%), Caucasians (80%) and overweight with BMI of 28.5 ± 5.9 kg/m^2, waist circumference was 35.93 ± 6.45 inches.

As there were many overweight patients and atenolol AUC_{0-24} was associated with body weight (r= -0.65, p=0.009) and BMI (r= -0.57, p=0.025), we normalized the atenolol AUC_{0-24} with respect to body weight (AUC_n). Atenolol AUC_{0-24} and AUC_n, glucose AUC_{0-2} and insulin AUC_{0-2} values are reported in Table 3-2. The atenolol pharmacokinetic estimates are reported in Table 3-3. The average atenolol AUC_{0-24} was 10,052 ng.hr/mL, the maximum concentration (C_{max}) achieved was 1195.7 ng/mL, time to achieve C_{max} was 2.6 hours with a half life of 6.4 hours, the apparent oral clearance (Cl/F) was 182.3 mL/min and apparent volume of distribution (Vd/F) was 100.0 liters which is consistent with previous literature (Brown et al., 1976; Mason et al., 1979). We also calculated glucose and insulin AUC_{0-2} during the 2 hour OGTT. The mean glucose AUC_{0-2} was 241.4 mg.hr/dL and mean insulin AUC_{0-2} was 119.8 µIU.hr/mL. The glucose and insulin levels during the 2 hour OGTT are shown in Figures 3-2a and 3-2b respectively. Lipid data was not available for 3 patients and insulin data was not available for 6 patients and so they were not included in the analysis. There were no statistical significant

changes in any of the metabolic parameters after 8 weeks of treatment. The changes in lipids and glucose, insulin after atenolol treatment are reported in Tables 3-4 and 3-5.

The association between the atenolol AUC_n and other variables including log glucose AUC, log insulin AUC, body weight, BMI, waist circumference, hip circumference was evaluated. We also looked if the change in triglycerides, other lipids, glucose and insulin levels after atenolol treatment were associated with atenolol AUC_n. Subsequently, we did a regression analysis in which we forced these variables in the model.

The correlation coefficients are reported in Table 3-6 and Table 3-7. There was no relation between atenolol AUC_n and glucose AUC (r= -0.078, p=0.78), however, there was a significant negative relation between atenolol AUC_n and insulin AUC (r= -0.69, p=0.0043). We did not find any relationship between atenolol AUC_n and (Δ) triglycerides (r=-<0.001, p=0.97), (Δ) glucose (r=0.26, p=0.42), (Δ) HDL (r=0.09, p=0.77), or (Δ) total cholesterol (r= 0.04, p=0.88). However, log atenolol AUC was inversely related to (Δ) insulin (r= -0.61, p= 0.047).

We performed a forward stepwise regression of atenolol AUC_n vs. log insulin AUC and (Δ) insulin. The analysis lost significance for atenolol AUC_n and log insulin AUC, (Δ) insulin once we adjust for other variables like weight and BMI (Table 3-8).

We observed four patients (# 3,11,14,15) who had plasma glucose levels above 140 mg/dL at the end of 2 hour OGTT and hence were classified as having impaired glucose tolerance according to World Health Organization's and American Diabetes Association's criteria (American Diabetes, 2008) (Figure3-2a). The mean atenolol AUC_{0-24} for these four patients was 8697 ng.hr/mL and mean glucose AUC_{0-2} was 303.6 mg.hr/dl and the mean atenolol AUC_{0-24} for rest was 10599.3 ng.hr/mL and mean glucose AUC_{0-2} was 218.79 mg.hr/dl. Two patients (# 5, 12) developed hyperinsulinemia, mean insulin AUC_{0-2} was 345.26 mU.hr/mL and

their mean atenolol AUC was 9195.8 ng.hr/mL (Figure3-2b). Patients 4, 7, 10, 14 and 15 had low insulin levels and low insulin AUC_{0-2} and had high atenolol AUC_{0-24} (Table 3-2). Their mean atenolol AUC_{0-24} was 13613.01 ng.hr/mL and mean insulin AUC_{0-2} was 45.92 μIU.hr/mL and mean atenolol AUC_{0-24} in remaining patients was 8331.86 ng.hr/mL and mean insulin AUC_{0-2} was 156.87 μIU.hr/mL.

Discussion

We hypothesized, the occurrences of the metabolic abnormalities with atenolol were concentration related and that people with higher plasma atenolol exposure are more likely to develop these AME. These short term AME have the potential to subsequently develop into new cases of type 2 diabetes, dyslipidemia.

Beta adrenergic receptor blockers decrease insulin sensitivity, an effect observed with nonselective beta blockers as well as selective beta blockers at higher doses when they lose their specificity and bind to beta 2 adrenergic receptors (Lundquist, 1971; Pollare et al., 1989b; Jacob et al., 1998). We did not find any association between atenolol AUC_n and glucose AUC_{0-2} and a significant negative correlation between atenolol AUC_n and insulin AUC_{0-2} and (Δ) insulin was lost in the regression model after adjustment for weight and BMI indicating that the association was with these variables. Similarly, we did not observe any association with atenolol AUC_n and change in triglyceride levels after atenolol treatment. This lack of association may be is the result of small sample size and short follow up of the patients.

We found patients who had delay in insulin response over the 2 hour OGTT, which can be due to decrease in insulin secretion (Lundquist, 1971; Pollare et al., 1989b; Jacob et al., 1998) and two patients (# 5, 12) who were hyperinsulinemic and had low glucose levels at the end of OGTT. Patients 4, 7, 10, 14 and 15 had low insulin levels and low insulin AUC_{0-2} during 2 hour OGTT and had high atenolol $AUC_{0-24;}$ however, this is not statistically significant. This can be

explained by the fact that the beta 2 adrenergic receptors located in the pancreas being blocked by atenolol at higher doses and thereby reduce insulin secretion (William-Olsson et al., 1979). Insulin is secreted in a biphasic manner on glucose exposure; there is an instantaneous first phase insulin secretion followed by second lag phase. Beta blockers decrease the first phase of insulin secretion leading to more insulin secretion during the second phase thereby causing hyperinsulinemia. None of these observations were statistically significant which may be due to a small sample size and other confounding variables.

Atenolol AUC_n and log glucose AUC were not associated with each other. Beta blockers have been found to increase blood glucose levels (Cerasi et al., 1972; Wright et al., 1979). We found four patients (# 3, 11, 14 and 15) who had impaired glucose tolerance. Their glucose levels remained higher during the 2 hour OGTT but they had normal insulin levels and atenolol AUC_n was not significantly different from the rest of the patients (American Diabetes, 2008).

There was no association between lipid changes after atenolol treatment and atenolol AUC_n. There are studies that have observed neutral or opposite effects of BB on the exposure on lipids (Eliasson et al., 1981; Weiner and Rossner, 1983). This might be due to short period of treatment or lack of dietary control in the study period. We did not have any restrictions on diet during the study period and we also did not maintain a food log or physical activity which can affect the lipid levels. Only one patient was on lipid lowering therapy (simvastatin), no other patient was on any other co medication which can potentially confound the observations.

In our study, patients were taking atenolol 50mg for 3-4 weeks, after which they took atenolol 100mg for additional 4 weeks, so in all these patients were on atenolol treatment for average 8 weeks. In this short period of time, we were not able to see the development of AME on fasting glucose and triglyceride levels in this small cohort but more abnormalities were

evident in the OGTT. There are no previous studies that have used plasma concentrations of BBs to correlate with the glucose and change in triglyceride levels.

We did not find plasma atenolol concentration is a risk factor for patients developing AME. However, our study has small sample size and our power was to detect a correlation coefficient of 0.62. It is possible that smaller correlations would be evident with a larger sample, although the data do not suggest this would be the case. Further studies should be conducted to determine the possible causes for development of AME because of BB treatment; if we can identify patients who will develop AME, we may be able to reduce the incidence of new onset diabetes in these patients. Our data do not suggest atenolol exposure is one of the important factors. If the factors that do place a patient at risk for the AMEs can be identified, subsequently these important anti-hypertensive agents can continue to be used in the remaining population without bias relative to other anti-hypertensive agents due to these AME.

Table 3-1. Clinical characteristics of patients (n = 15)

Age, years (mean ± SD)	46±8.9
Gender: Men /Women (%)	40/60
Race: Caucasians/ African Americans (%)	80/20
Systolic blood pressure, mm Hg	133.13±13.17
Diastolic blood pressure, mm Hg	81.86±6.79
BMI ,kg/m^2	28.5±5.9
Waist circumference ,inches	35.93±6.45
Hip circumference, inches	38.21±12.09
Fasting glucose, mg/dL	81.8±7.79
Fasting insulin, μIU/mL	8.26±7.5
Fasting triglycerides, mg/dL	149.93±84.97
Fasting HDL-cholesterol, mg/dL	50.46±19.18
Fasting Total cholesterol, mg/dL	191.73±33.85
HOMA-IR	1.73±1.56
HOMA-BC	182.75±143.04

Data are expressed as mean ±SD

Table 3-2. Atenolol AUC and glucose, insulin AUC during OGTT

Patient #	ATN AUC_{0-24}, ng.hr/mL	Log ATN AUC_{0-24}, (AUC_n) ng.hr/mL.kg	Glucose AUC_{0-2}, mg.hr/dL	Log glucose AUC_{0-2}, mg.hr/dL	Insulin AUC_{0-2} µIU.hr/mL	log Insulin AUC_{0-2} µIU.hr/mL
A01	7213.9	4.64	230.7	5.44	104.79	4.65
A02	7746.6	4.42	190.7	5.25	87.94	4.48
A03	6211.6	4.28	326.7	5.79	179.26	5.19
A04	13026.1	5.16	209.5	5.34	36.61	3.60
A05	8969.6	4.41	268.3	5.59	280.47	5.64
A06	9127.8	4.76	202.5	5.31	113.65	4.73
A07	15189.1	5.55	268.7	5.59	28.86	3.36
A08	12345.9	5.09	161.5	5.08	128.33	4.85
A09	8060.1	4.7	224.5	5.41	48.14	3.87
A10	16434.0	5.63	193.3	5.26	43.54	3.77
A11	5163.7	3.76	241.3	5.49	156.63	5.05
A12	9422.1	4.57	214.5	5.37	410.06	6.02
A13	9057.2	4.82	242.5	5.49	59.46	4.09
A14	10675.3	5.17	276.5	5.62	49.69	3.91
A15	12740.6	5.21	369.7	5.91	70.84	4.26
Mean	10092.3	4.81	241.4	5.46	119.88	4.55
SD	3253.7	0.5	54.3	0.22	104.67	0.77
CV	32.3	9.61	22.5	3.94	87.31	17.01
Minimum	5163.7	4.28	190.7	5.25	36.61	3.36
Maximum	16434.0	5.17	369.7	5.91	410.06	6.02
Fold diff.	3.2	1.21	1.9	1.12	11.20	1.79

Table 3-3. Pharmacokinetic estimates of atenolol

Patient #	C_{max}, ng/mL	$T_{1/2}$, hours	T_{max}, hours	Vd/F, liters	Cl/F, mL/min
A01	943.3	7.1	1.5	141.7	231.67
A02	1128.6	6.3	2.0	118.2	215.00
A03	1034.7	4.7	3.0	110.3	268.33
A04	1182.8	7.3	4.0	81.2	128.33
A05	912.1	6.8	2.0	110.2	185.00
A06	1100.4	6.4	2.0	101.0	183.33
A07	1506.1	6.7	4.0	63.6	110.00
A08	1448.6	6.0	4.0	70.0	135.00
A09	1051.6	4.8	3.0	86.5	206.67
A10	1255.4	10.7	3.0	93.9	101.67
A11	577.1	6.8	1.5	191.5	323.33
A12	1359.1	5.7	3.0	87.7	176.67
A13	1326.4	4.7	3.0	75.8	183.33
A14	1751.9	7.2	2.5	97.0	156.67
A15	1233.2	6.4	1.5	71.9	130.00
Mean	1187.5	6.5	2.6	100.0	182.33
SD	280.9	1.5	0.9	32.7	60.77
CV	23.6	22.1	33.7	32.7	33.32
Minimum	577.5	4.7	1.5	63.6	101.67
Maximum	1751.9	10.7	4.0	191.5	323.33
Fold diff.	3.1	2.3	2.7	3.0	3.20

Table 3-4. Changes in lipids after atenolol treatment

	Triglycerides, mg/dL (n=12)	HDL, mg/dL (n=12)	TC, mg/dL (n=12)
Baseline	133.10 ±88.13	46.12±17.07	200.91±34.69
After 8 weeks	133.87±65.38	48.66±20.95	201.83±38.71
Change	0.87±55.98	2.53±6.4	0.91±21.81

Data are expressed as mean ±SD, groups compared by paired t-test, no statistical significant change in any variable

Table 3-5. Changes in glucose, insulin after atenolol treatment

	Glucose, mg/dL (n=11)	Insulin, μIU/mL (n=9)	HOMA-IR (n=8)	HOMA-BC (n=8)
Baseline	83.72±8.20	8.36±5.09	1.79±1.23	143.17±59.18
After 8 weeks	84.45±6.68	9.14±6.26	2.07±1.34	160.32±102.77
Change	1.90±6.18	0.77±3.2	0.28±0.6	17.13±75.0

Data are expressed as mean ±SD, groups compared by paired t-test, no statistical significant change in any variable

Table 3-6. Pearson's correlation between atenolol AUC_n and glucose AUC, insulin AUC

Variable	Atenolol AUC_n	Log insulin AUC	Log glucose AUC
Atenolol AUC_n	1	r= - 0.69, p= 0.004	r= -0.078, p=0.78
Log insulin AUC	r= - 0.69, p= 0.004	1	r=0.004, p=0.98
Log glucose AUC	r=-0.078 p=0.78	r=0.004, p=0.98	1

Table 3-7. Pearson's correlation between atenolol AUC_n and changes in lipids, glucose

Variable	Δ Triglycerides	Δ glucose	Δ insulin	Δ HOMA-IR	Δ HOMA-BC	Δ HDL	Δ TC
Atenolol AUC_n	r=-0.01, p= 0.97	r=0.26 p=0.42	r=-0.67, p=0.04	r=-0.49, p=0.21	r=-0.59 p=0.11	r=0.09 p=0.77	r=0.04 p=0.8

Table 3-8. Multivariate analysis

Dependent variable	Covariate entered	Estimate	Standard error	p-value
Log insulin AUC	Atenolol AUC_n	-1.03	0.32	0.008
Log insulin AUC	Atenolol AUC_n	-0.32	0.35	0.38
	BMI	0.087	0.03	0.014
Δ insulin	Atenolol AUC_n	-0.42	3.36	0.90
	Weight	0.13	0.09	0.22

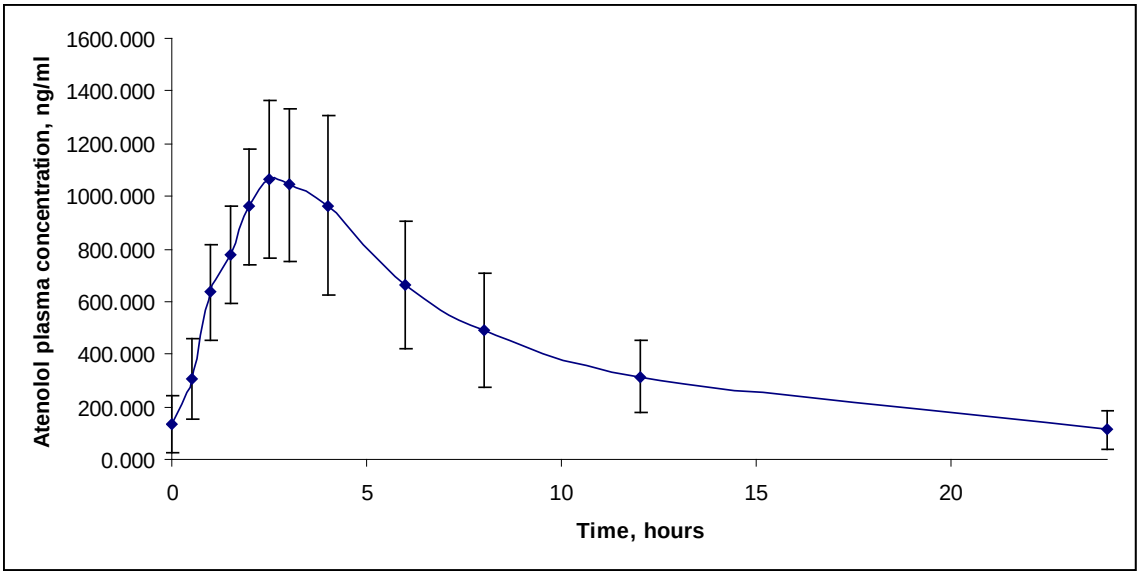
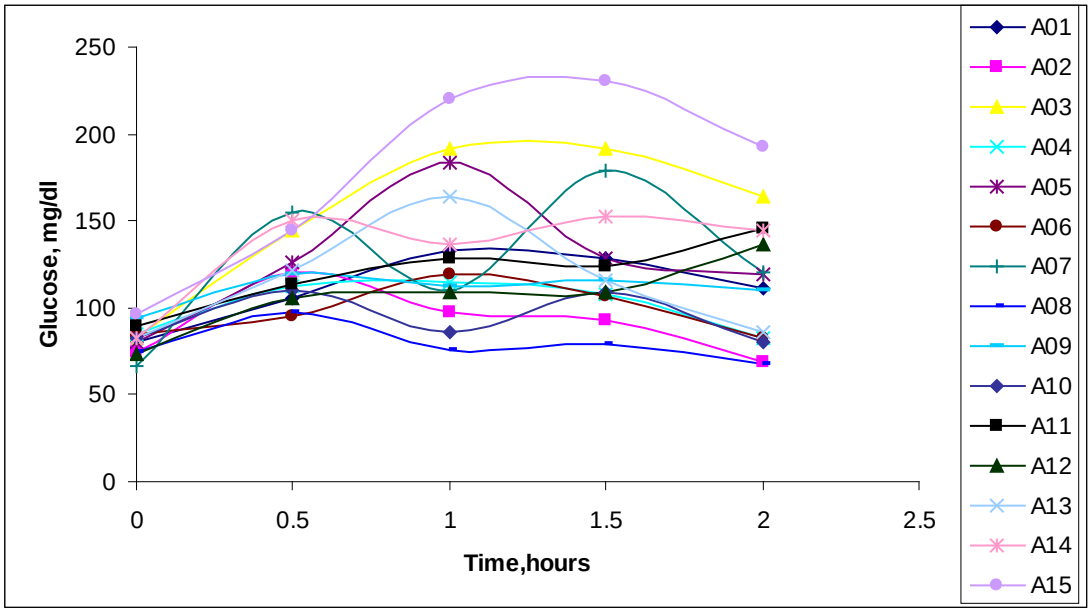


Figure 3-1. Atenolol plasma concentration (ng/mL) vs. time (hours) profiles (mean ±SD) after oral administration of 100 mg atenolol once a day

A)



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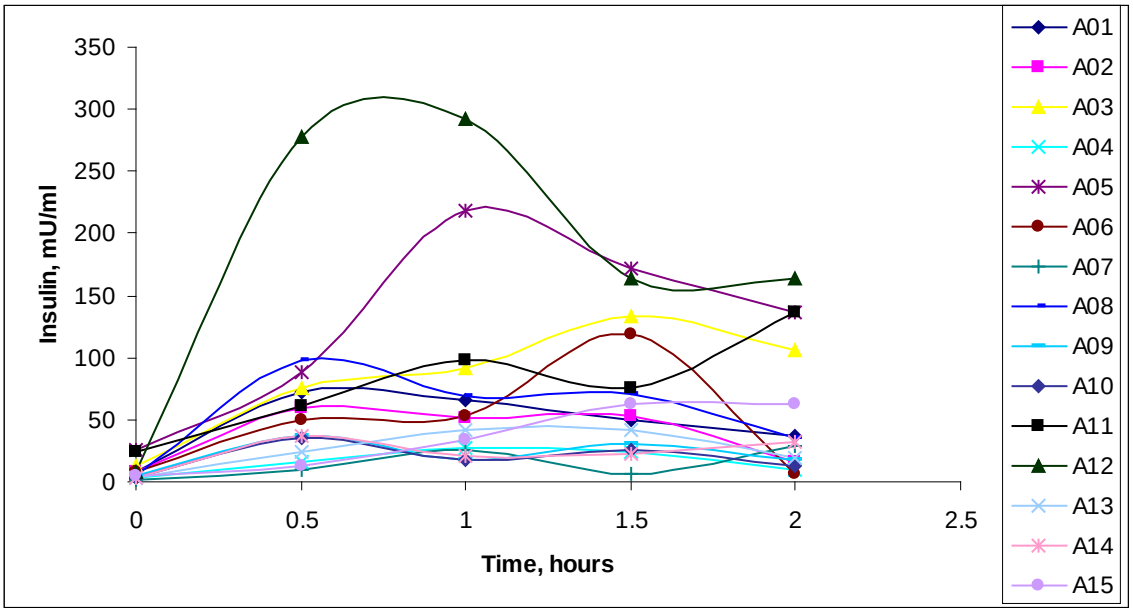
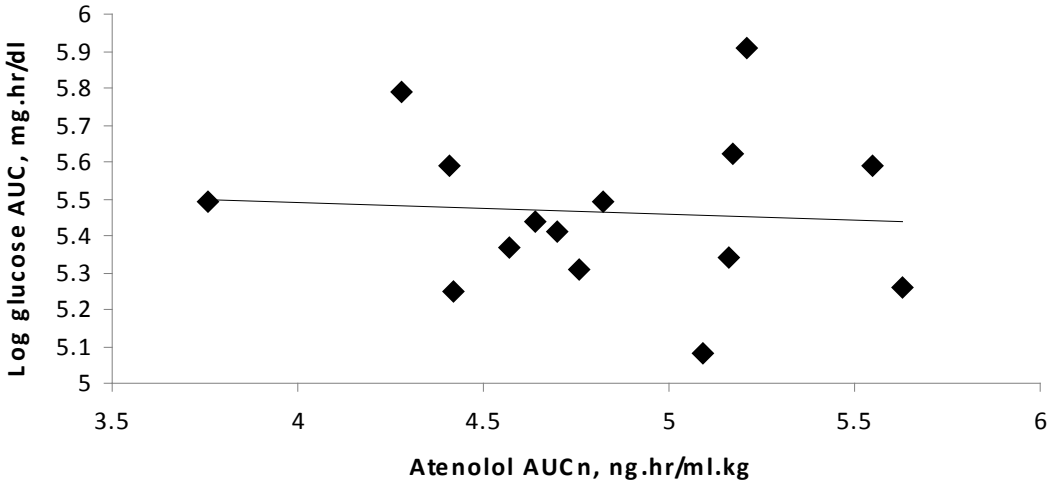


Figure 3-2. Plasma Levels During OGTT A) Glucose B) Insulin

A)



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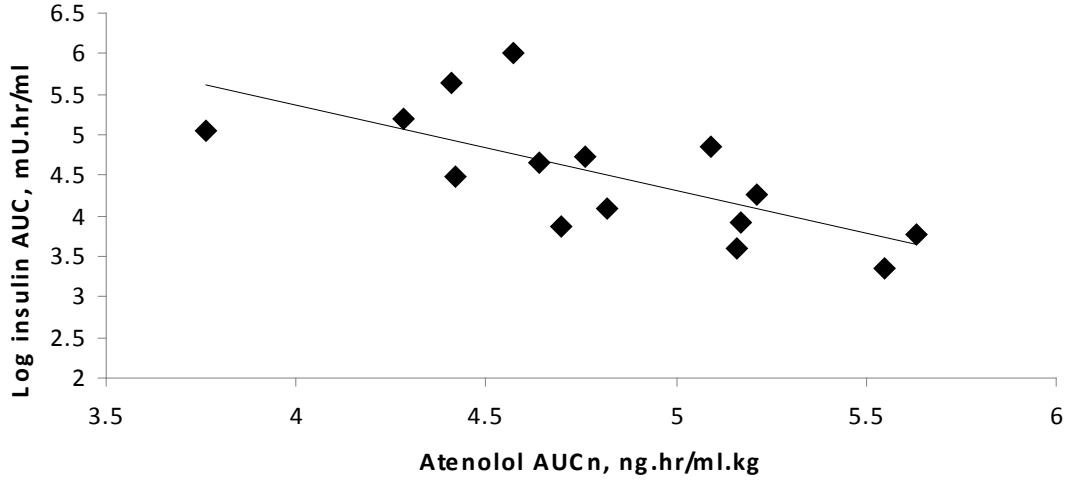

Figure 3-3. Correlation Coefficients (r) between A) atenolol AUC_n and glucose AUC_{0-2}, B) atenolol AUC_n and insulin AUC_{0-2}

CHAPTER 4
CONCLUSIONS AND FUTURE DIRECTIONS

CVD is leading cause of death in the world and HTN is one of important risk factors for CVD. There are several classes of anti-hypertensive agents used clinically to control HTN. BBs have been widely used for several years for their safety and efficacy in reducing mortality and morbidity. However, there have been several concerns with the use of BB as first line therapy in HTN over the new agents in development of AME on glucose, insulin and lipids. However, unlike the reports on low dose thiazide diuretics to have neutral effect on development of these AME, there are conflicting studies on BB associations with AME.

We had hypothesized occurrence of the AME are related with degree of atenolol exposure, and patients with higher atenolol AUC will be more likely to develop the AME. However, we did not found any association between plasma atenolol concentrations and glucose levels during the 2 hour OGTT or change in triglycerides, change in glucose levels and between plasma atenolol concentrations.

There are several key points to note from this study. The occurrence of AME on BB therapy is a major factor in the preference of other anti-hypertensive agents over BB and we were not able to associate plasma drug levels of atenolol with the AME. This leaves the question unanswered of why BBs are associated with AME and though there is high variability in the PK parameters as seen in our study as well as other studies, we cannot associate this PK variability in the development of AME. Thus, further studies to explore the reasons for development of AME by BBs like atenolol are needed. Ultimately, we can avoid or prevent the occurrence of AME, these important anti-hypertensive agents can still be safely used rather than being rejected totally.

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BIOGRAPHICAL SKETCH

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