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# Transdermal Formulation Development and Topical Administration of Atenolol to Cats

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## Abstract

Atenolol diffusion through synthetic membrane, cloned human epidermis, and cat ear skin was performed utilizing a Franz diffusion cell. Transdermal drug diffusion enhancers' ethanol, glycerol, propylene glycol, polysorbate 80 and Dimethyl isosorbide (DMI) were added to the topical formulations and tested for their ability to enhance drug permeation through the test membranes. Topical formulation with penetration enhancers showed a rapid burst of atenolol diffusion for the first two hours (35.5 to 40 μg/ml) followed by a zero-order sustained diffusion of 2.7 μg/cm$^2$/h of atenolol for up to twenty-four hours after application to test membranes. Increased atenolol flux through different test membranes was greatest for synthetic membrane. The topical application of the optimized atenolol formulation to cat skin containing permeation enhancers aided transdermal atenolol drug delivery to treat cats with hypertrophic obstructive cardiomyopathy. The optimum topical formulation demonstrated two fluxes through cat skin, the burst flux (15.7 μg/cm$^2$/h) and a sustained flux (2.7 μg/cm$^2$/h). Measured atenolol concentrations in cats at 3, 6 and 12 hours after transdermal atenolol application were 432.7 ng/ml ± 323.3, 262.4 ng/ml ± 150.1, and 253.3 ng/ml ± 133.6 respectively. Six of 7 cats achieved therapeutic serum atenolol levels (200 ng/ml) for at least one time point. Five of 7 cats had therapeutic serum atenolol concentrations 3 hours post-atenolol. At the 6 hours post-atenolol time point, Four had a therapeutic serum atenolol concentration while at 12 hours post-atenolol dosing, 4 of 7 cats had therapeutic serum atenolol concentrations. Transdermal atenolol administered at 25 mg q12h resulted in clinically therapeutic serum atenolol concentrations in the majority of healthy cats. The optimum transdermal formulation enabled good drug delivery feasible for transdermal application in a clinical trial in cats.

## Keywords

Atenolol, Transdermal Delivery, Formulation, Cats

## 1. Introduction

Atenolol is a beta one receptor antagonist commonly prescribed in cats affected with hypertrophic obstructive cardiomyopathy. The theoretical benefits of effective beta one blockade include decreased myocardial oxygen demand, reduced or abolished left ventricular outflow tract obstruction and heart rate reduction with increased diastolic filling time [1]. In addition, atenolol is used to treat animals with high blood pressure.

Atenolol tablets are used for treatment of hypertrophic cardiomyopathy (HCM), left ventricular outflow tract obstruction and hypertension secondary to hyperthyroidism [1]. In veterinary medicine, atenolol like many other drugs is not FDA approved for use in animals and is not available from a veterinary pharmaceutical manufacturer. Instead, veterinarian's use atenolol drug products prepared by a compounding pharmacy or FDA approved tablets for human use. Cats with heart disease receive an atenolol dose of 6.25 to 12.5 mg once or twice a day, usually tablets. Administration of atenolol has not proven to change the outcome in affected cats. However, many veterinary cardiologists prescribe atenolol for dynamic left ventricular outflow tract obstruction using highly bioavailable oral tablets [2].

Administration of oral tablets to cats can be a challenging issue (scratching, biting, avoidance, etc.) for owners leading to skipping or not adequately dosing their animals producing sub-therapeutic drug concentrations resulting in a lack of therapeutic compliance in cats and not maintaining therapeutic drug concentrations [3]. Therefore, there is an increasing demand to develop an alternative route of administration for atenolol to cats. In human, billions of transdermal drug dosage forms for several drugs are produced every year. Thus, clinical experience suggests use of transdermal drug formulations to administer atenolol for cats may be beneficial. Unfortunately, not every available drug can be a substrate for transdermal formulation.

A study conducted on 13 cats diagnosed with hyperthyroidism was treated with a transdermal methimazole topical formulation that was applied to the internal ear pinna at a dose of 5 mg. This prospective clinical study suggested that transdermal methimazole is an effective and safe alternative to the conventional oral formulation [4]. Another study found similar results that transdermal application of methimazole used as alternative dosage form for therapy in cats was efficacious [5].

The pharmacokinetics and pharmacodynamics of atenolol given as a single dose intravenously and orally to cats was performed [6]. Post administration at 6 and 12 hours resulted in significant decreases in heart rate following isoproterenol challenge in all cats with a minimum atenolol plasma concentration of more than 260 ng/ml. Another small study compared atenolol pharmacodynamics after oral and transdermal administration in healthy male cats. Cats receiving oral atenolol reached therapeutic concentrations after oral administration, whereas cats receiving the transdermal formulation had atenolol concentrations below

260 ng/ml at the same time point [7]. These results raise questions specifically regarding the direct substitution of transdermal doses at equivalent oral doses, and even possible variability of compounded medications. This project seeks to develop a transdermal formulation to facilitate atenolol diffusion across cat skin for therapeutic efficacy. Therefore, the aim of this study is to address the problem of the formulation of atenolol for transdermal delivery by optimizing the concentration of drug within the carrier gel. Secondly, this study aims to assess serum atenolol concentration and therapeutic response as it relates to dose and plasma concentration.

## 2. Materials and Methods

Atenolol 100 mg tablets were obtained from TEVA (Gardna CA); Ethanol USP Grade from Pharmaco, AAPER from Brookfield. C. T. New York, 10013; Dimethyl isosorbide (DMI) from CRODA (NJ, USA 300 Columbus Cir # A, Edison, NJ 08837). Glycerol from EMD chemicals Inc (110 EMD Blvd, Port Wentworth, GA 31407 (USA), Propylene glycol and Triethanolamine from J. T. Baker Inc (600 Broad St, Phillipsburg, NJ 08865 USA). Polysorbate 80 obtained from Acumedia (620 Lesher Pl, Lansing, MI 48912. Houston Texas, USA) and Carbomer 934 obtained from Spectrum (V Gardena, CA 1434 W Gardena Blvd, Gardena, CA 90247).

Membranes filters used were transfer medium pure nitrocellulose (0.45 um) Trans-Blot obtained from BIO-RAD, (4000 Alfred Nobel Dr, Hercules, CA 94547, USA). Cloned human epidermis was obtained from MatTek Corporation (200 Homer Ave, Ashland, MA 01721). Fresh cat skin was obtained from Oregon State University, College of Veterinary Medicine, Cardiology department, Necropsy Laboratory.

### 2.1. Preparation of Atenolol Formulations

Development of topical atenolol formulation: different formulations were produced by changing the percentage of the ingredients. The next step was testing atenolol diffusion through various membranes to select the optimal formula for the in vivo study phase. Different atenolol topical formulations containing different percentages of ingredients that were prepared and subjected to *in vitro* permeation of atenolol across synthetic membrane, cloned human epidermis and freshly prepared cat skin are shown in **Table 1**.

### 2.2. The Selection of the Gel Ingredients

The gel contains propylene glycol and glycerin as humectants, co-solvents and pharmaceutical excipients. A surfactant (Polysorbate 80) was added to aid in emulsification of atenolol and to improve drug transportation. Ethanol also was added to improve the skin permeation of atenolol. Dimethyl isosorbide (DMI) was used as a drug carrier and transdermal enhancer. The gel base was either 1% or 0.75% carbomer that was neutralized with 0.45% tri-ethanolamine to produce

**Table 1.** Composition of various Atenolol formulations (g), all formulations contain 0.45% triethanolamine and either 1% carbomer (F7, F8, F9) or 0.75% carbomer (F1, F2, F3, F4, F5, F6).

| Formulation Code | Percent Atenolol | Percent Tween 80 | Percent Glycerol | Percent Propylene glycol | Percent Ethanol | Percent DMI |
|---|---|---|---|---|---|---|
| F1 | 1 | 5 | 5 | 15 | 5 | 0 |
| F2 | 1 | 5 | 10 | 15 | 5 | 0 |
| F3 | 1 | 5 | 15 | 15 | 10 | 0 |
| F4 | 1 | 5 | 15 | 15 | 10 | 5 |
| F5 | 1 | 5 | 15 | 15 | 10 | 10 |
| F6 | 1 | 5 | 15 | 15 | 10 | 15 |
| F7 | 1 | 2.5 | 15 | 15 | 5 | 5 |
| F8 | 1 | 2.5 | 15 | 15 | 10 | 10 |
| F9 | 1 | 2.5 | 15 | 15 | 10 | 15 |

a pH of 7. Atenolol formulations were prepared using different concentrations of different enhancers (Table 1).

## 2.3. Gel Formulation

Fifty grams of a clear (1% atenolol gel) was prepared as follows. The gel was formulated by adding 7.5 g glycerin to the 7.5 g propylene glycol and then mixing with water to make a dispersion. Atenolol was added to this solution followed by the 2.5 g surfactant (Polysorbate 80) and 7.5 g DMI. Carbomer 0.375 g and 0.225 g triethanolamine were dispersed in water in another beaker. The two mixtures were thoroughly mixed together via trituration forming a gel. The gel was stored at room temperature after preparation 22˚C to 25˚C [7].

## 2.4. Instrumentation

A simple and effective High Performance Liquid Chromatography method was developed and validated to determine atenolol concentrations in various types of samples including cat's plasma. Atenolol was analyzed using HPLC-UV detection. Chromatographic analysis was carried out using a Shimadzu Prominence High performance liquid chromatographic system (Kyoto, Japan) LC-2010A HT model and equipped with a dual UV detector and pump.

All reagents and solvents were HPLC grade. Methanol and acetonitrile were purchased from Fisher Scientific (755 US-202, Branchburg, NJ 08876; USA). Glacial acetic acid which is used to produce a pH of 3 in the mobile phase was obtained from VWR international Philadelphia (PA 2039 Center Square Rd. USA).

The HPLC analysis was performed using a 4.6 × 150 mm column (Kinetex, Phenomenex (411 Madrid Ave, Torrance, CA 90501 CA, USA) packed with 5-µm C-18 chromatographic medium and connected to a pre-column (security

guard 2.1 × 4.6 mm, Kinetex Phenomenex, CA, USA). The flow rate was 1 ml per minute and the detector wavelength was set at 230 nm with the column temperature fixed at 25˚C. The injection volume was 20 µl, and the run time was 5 minutes. Atenolol was eluted out at retention time of 2 min [8].

The first part of this study is the creation of a HPLC atenolol calibration curve. Serial dilutions of 1mg/ml stock solution of atenolol were performed to prepare different concentrations starting from 40, 20, 10, 5, 2.5, 1, 0.75, 0.5, to 0.1 (µg/ml) to run through the HPLC (Shimadzu). The mobile phase consists of 60:20:20 acidic water, acetonitrile and methanol and sonicated for one hour before use. The mobile phase was made acidic pH 3 by the addition of few drops of glacial acetic acid. The flow rate is 1 ml/minute. The corresponding AUC of atenolol HPLC peaks produced by the prepared atenolol standard solutions were plotted versus the atenolol standard concentrations that produced them and a calibration curve was prepared. Validation of the calibration curves was done by performing three sequential HPLC runs that produced equivalent standard calibration curves. Validation of the HPLC assay method was performed according to FDA recommendations for assessment. The assay validations of the HPLC methods included the assay's linearity [8] [9].

## 2.5. Linearity

Upon plotting the chromatograms average ratio of peak areas (AUC) of Atenolol versus concentration (range of 0.1 - 40 µg/ml) produced a straight line. Linear regression on the calibration curve was applied to obtain the equation ($y$ = 157,025$x$ + 48,772) and $R^2$ = 0.9976. ANOVA test (one way) was used to compare the three calibration curves. Not statistical difference was observed between the three runs ($p < 0.05$). The relationship for the method was clearly linear based on the high $R^2$ (Correlation coefficient). The lower limit of detection (LOD) that could be detected (LOD) was 0.045 µg/ml while the limit of quantification (LOQ) of atenolol was 0.1 µg/ml. Linear relationship between the peak ratios and atenolol concentrations in the range of 0.1 to 40 µg/ml with $R^2$ = 0.9976.

## 2.6. *In Vitro* Skin Permeation Study

Atenolol Permeation studies through synthetic membrane, cloned human epidermis [8], and cat skin were conducted using Franz diffusion cells; PermeGear vertical glass diffusion cells from HAAK-L. Six Franz diffusion cells (Hanson Research Chatsworth CA USA), with a surface area of 6.43 $cm^2$ and a receptor cell volume of 14 ml were used. The receptor solution was a phosphate buffer solution (PH 7.4) stirred at 600 rpm and maintained at 37˚C by use of a heated circulating water heater with pump delivering heated water to each chamber. The membrane or skin initially was allowed to hydrate in the Franz Cells for an hour. During this time the cells were occasionally turned upside down in order to allow the escape of any air bubbles that might develop on underside of the membrane or skin. Then, each membrane or cat's skin was treated with one

gram of the selected test formulation. A control experiment was also run in which the membrane or skin was treated with a one percent solution of atenolol.

### 2.7. Sampling

Drug permeation through test membranes was allowed to continue for 24 hours. At chosen times, a 100 µl volume of solution was withdrawn from each Franz cell receiver solution and replaced with the same volume of phosphate buffer solution to compensate for volume loss. The samples withdrawn were analyzed by direct injection into the HPLC. Atenolol concentrations were determined from the prepared calibration curve of standard atenolol solutions. In all cases, atenolol concentration values obtained for each aliquot was corrected for the progressive dilutions that occur during the course of the Franz diffusion cell experiment [10].

The test membranes were mounted between the donor and receptor compartments. The effective diffusion area of the cell was 6.43 $cm^2$ and with a receptor volume of 14 ml. The receptor medium was phosphate saline buffer pH 7.4. The prepared atenolol gel was placed on one side (upper side) of the membrane. The receptor compartment was bounded by a water jacket to maintain the temperature at 37˚C. The receptor fluid was stirred by a Teflon-coated magnetic stirrer [11]. At specific time intervals, samples in the acceptor chamber were collected at 0, 2, 4, 6, 8, 12, 24 hours and replaced with an equal volume of receptor media for drug content determination. Drug concentration was determined using HPLC.

Selected atenolol formulations were applied to necropsy skin samples of the pinna of cats. After removal of epidermal hair, the skin was cleansed and any adhering subcutaneous tissue and blood vessels were removed. The excised cat skin placed between the half cells of the Franz-Chin diffusion cells to measure atenolol permeability through cat skin.

### 2.8. *In Vivo* Study

Seven adult healthy cats presented to the OSU Cardiology service were included in the in-vivo study. The cats were all mixed breeds and had an age range of 1.3 to 8.5 years. All study participants underwent a physical examination, blood pressure measurement, electrocardiogram, and echocardiography. To ensure appropriate renal and hepatic function, baseline blood work was also performed. Informed consent was obtained from all owners. The study was approved by the Oregon State University and the College of Veterinary Medicine's Institutional Animal Care and Use Committee. Cats showing normal screening values were considered healthy and included in the study.

The enrolled cats received concentrated transdermal atenolol (12.5 mg/0.1mL) on the inner pinna of alternating ears according to the following schedule: 12.5 mg once daily for 2 days, then 12.5 mg every 12 hours for 2 days, then 25 mg in the morning followed by 12.5 mg 12 hours later, then 25 mg every 12 hours for 3

days. On the 10th day of treatment, 25 mg of transdermal atenolol was applied in the morning. Then cats were admitted to the small animal veterinary hospital at OSU for participation in the study. Blood samples for measurement of serum atenolol concentration were collected at 3, 6, and 12 hours after drug administration. Blood samples were analyzed by an outside laboratory in Oklahoma by HPLC ms/ms. A 7-lead ECG was also performed at the 3, and 6 hour time points right after phlebotomy and an average heart rate (HR) was measured from a 30-second recording. Atenolol was not administered to any cat in the evening of hospitalization. A tapering dosage schedule consisting of 25 mg once daily for one more day, followed by 12.5 mg once daily for 2 days, then the treatment was discontinued.

Transdermal atenolol gels were packaged in syringes to make it easy to administer and dispense proper dosages. The animal's dose was put into the smallest amount of gel, usually 0.1ml containing 12.5 mg. The transdermal gel was applied to the animal's inner pinna.

### 2.9. Pharmacokinetics Analysis

A simulation convolution approach for a pharmacokinetic model utilizing an initial burst flux (40% of dose) of atenolol for administration (553 μg) followed by a zero-order flux (2.7 μg/hours) of ate1nolol across cat skin beginning 3 hours after atenolol topical application. Elimination of atenolol was assumed to be by a first-order process. Pharmacokinetic parameters were developed by closeness of fit of simulated data to actual data.

Simulation Convolution Pharmacokinetic Model:

$$C(t) = f_1(C_1 t) + f_2(C_2 t) - f_3(C_1 t)$$

where $f_1(C_1 t)$ is the burst effect absorption function.

$f_2(C_2 t)$ is the zero-order sustained release absorption function;

$f_3(C_1 t)$ is a first-order elimination process function.

Estimation of the pharmacokinetic parameters of transdermal atenolol was estimated to produce the half-life, clearance, volume of distribution and the elimination rate constant. Similarity factor $f_2$ and two fluxes (burst flux and zero-order fluxes) were identified for each concentration time graph.

**Statistical Analysis of Atenolol Pharmacodynamics**

Based on the small sample size, the data was considered nonparametric. The plasma atenolol concentration was evaluated using linear modeling by Spearman's rank correlation coefficient. The difference in baseline HR to stress provocation was assessed using the Wilcoxon matched-pairs signed rank test for each cat. Values of $p < 0.05$ were considered significant.

## 3. Results

*In vitro* drug release studies: The diffusion of atenolol through synthetic membrane from formulations 1 to 6 is presented in **Figure 1**. Formulation 3 showed rapid release and diffusion of atenolol through the cellulose nitrate membrane.

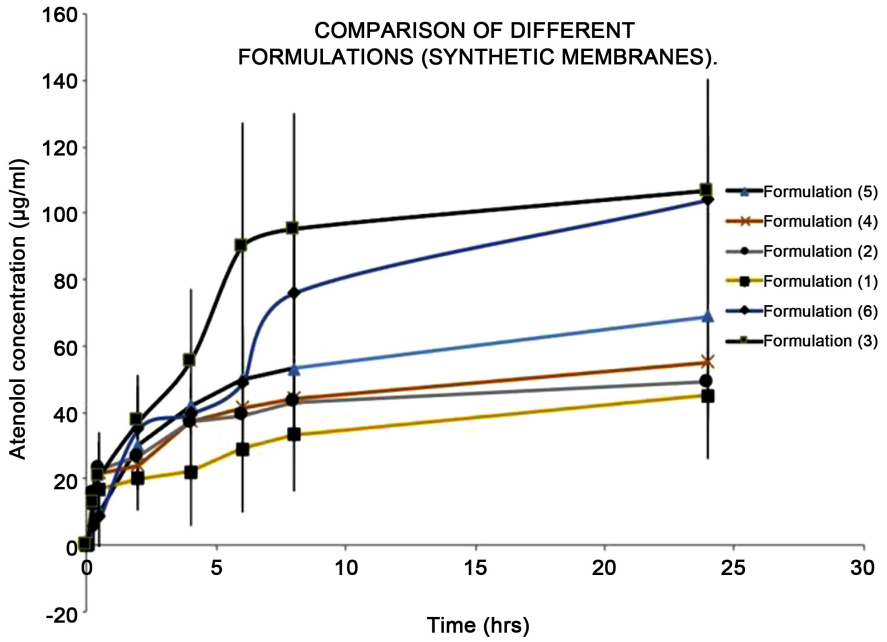


**Figure 1.** The permeation of atenolol through synthetic membrane.

Almost 90 µg/ml was measured in the receptor solution after six hours and 106.7 µg/ml at twenty fours. Formulation 6 in comparison released less atenolol at 6 hours and 24 hours (48.75 µg and 104.1 µg/ml, respectively).

Atenolol permeation through the cloned human epidermis was much less than that of synthetic membrane cat skin after 8 hours. Atenolol concentrations in the receptor compartment after 8 hours was 5.05 µg/ml ± 2.921, 5.878 µg/ml ± 3.868 and 16.26 µg/ml ± 5.842 for formulations 3, 5, and 6 respectively through cloned human skin.

In **Figure 2**, atenolol diffusion through freshly obtained cat skin for the three selected formulations showed that formulation 6 with 15% DMI had the highest drug permeation over 24 hours (76.56 µg/ml ± 20.44) followed by formulation 5 at 56.1 µg/ml ± 10.01 (with 10% DMI) and lastly formulation 3 at 45.61 µg/ml ± 9.26 with 0% DMI.

Atenolol exhibits two phases of diffusion through cat skin with different fluxes. The first flux is a Burst Flux which gave the following values: 7.67 to 56.73 (µg/cm$^2$/h) for Formulation 3. For Formulation 5 the Burst Flux (J1) ranged from 13.24 to 74.93 (µg/cm$^2$/h) and from 16.516 to 75.07 (µg/cm$^2$/h) for Formulation 6.

The second flux is consistent and shows a sustained release of atenolol. The second (sustained) Flux J2 ranged from 0.97 to 1.98 (µg/cm$^2$/h) for Formulation 3. The sustained flux for formulation 5 ranged from 1.19 to 2.48 (µg/cm$^2$/h) while that for Formulation 6 has the following range 1.07 to 3.5 (µg/cm$^2$/h).

Several models were tested to determine the mechanism of drug diffusion through cat and the various membranes [12] [13]. **Figure 3** gives the best model to explain atenolol diffusion through the membranes tested. The model was

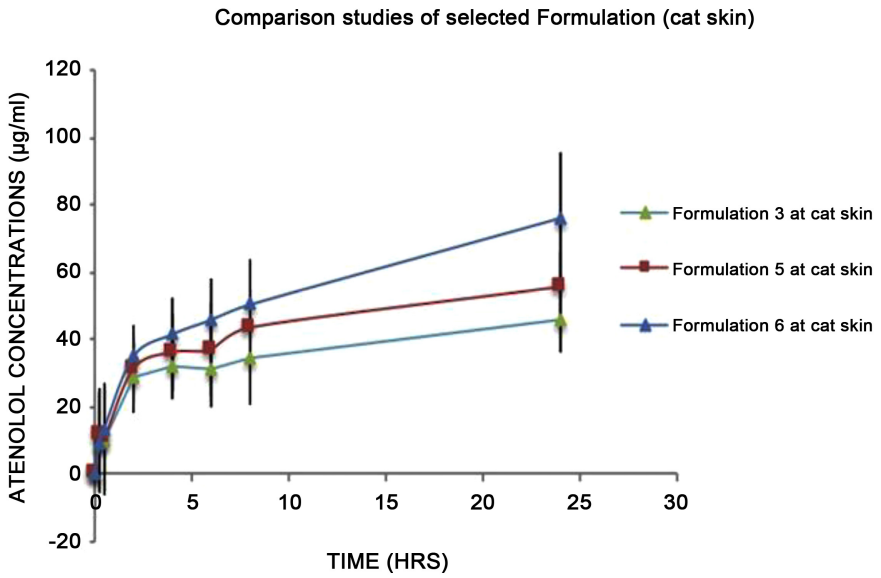


**Figure 2.** The permeation of atenolol through cat skin.

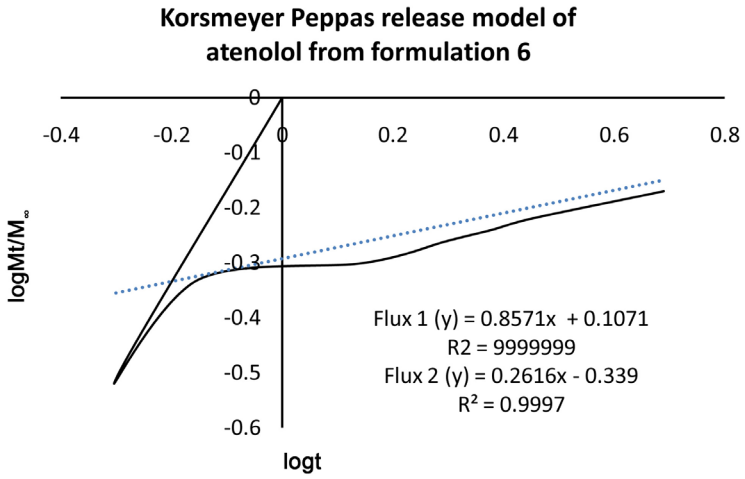


**Figure 3.** A Korsmeyer-Peppas plot of the formulation 6 permeation through cat skin. Axis are ($logMt/M_{\infty}$) versus log time.

especially capable in deciphering the type of diffusion seen through cat skin. Permeation of atenolol through cat skin followed a biphasic diffusion process. The initial diffusion was a burst of atenolol penetration through the membrane followed by a slower rate of drug permeation. Both phases of drug penetration followed a zero-order kinetic release pattern. The Korsmeyer-Peppas n value for the first phase was 0.8571 for the initial burst flux indicating a fickian diffusion process of a drug solution. The n value for the second flux phase was 0.2616. This indicates that dissolution of the drug from the drug particle occurs before diffusion through the cat skin. Erosion of the drug particle over may also be involved in this process.

The aid that the percutaneous drug enhancers have on atenolol diffusion through cat skin is presented in Figure 4. Atenolol solution applied to cat skin

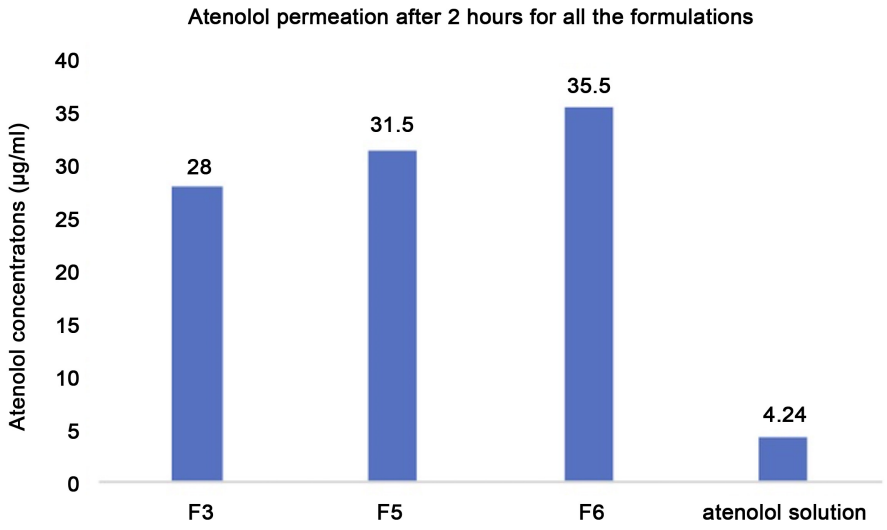


**Figure 4.** Atenolol permeation through cat skin for 2 hours compared to an aqueous solution of atenolol.

had very low permeability through cat skin compared to formulations 3, 5, and 6. Formulations 3, 5, and 6 increased penetration of atenolol through cat skin by 6.8, 7.4, and 8.3 times greater than atenolol solution alone.

The solubility of atenolol is 26.5 mg/ml in water. The solubility of atenolol was determined to be slightly greater than 100 mg/ml that is 4 times greater in solubility in the co-solvent system of Formulation 6 than water. The increase in atenolol solubility in the co-solvent does not fully account for the increase in permeation of atenolol through cat skin revealing that the use of penetration enhancers is needed to produce sufficient atenolol penetration.

A simulation of atenolol serum concentration was performed using a pharmacokinetic model where the absorption phases were the two diffusion fluxes through cat skin with a first-order elimination process. The data represented by the orange squares and the curved line through them in **Figure 5** was generated by using the pharmacokinetic parameters from the Quinones (1996) paper and observed fluxes calculated for formulation 6 in cat skin (burst flux 15.7 µg/cm$^2$/h for three hours and followed by a second flux of 2.7 µg/cm$^2$/h). The pharmacokinetic model was then applied to the observed cat serum concentrations determined after applying formulation 6 to cat skin. The triangles with the blue curved line were obtained from fitting the model to the observed cat atenolol serum data. The initial burst flux increased by 26.28% above the flux measured during the *in vitro* cat skin test. Flux 2 in the fitted model to the observed cat serum atenolol concentration from topical application to their ears was essentially the same as observed in the *in vitro* diffusion test through cat skin. The average pharmacokinetic parameters of half-life 3.45 h ± 0.5, $K_{el}$ 0.2 hr$^{-1}$ ± 0.3, clearance 260 ml/h/kg ± 72 and volume of distribution 1090 ml/kg ± 148 using the observed serum concentration values of 432.7 µg/ml at 3 hours, 262.43 µg/ml at 6 hours and 253.29 µg/ml at 12 hours, was essentially equivalent to the values

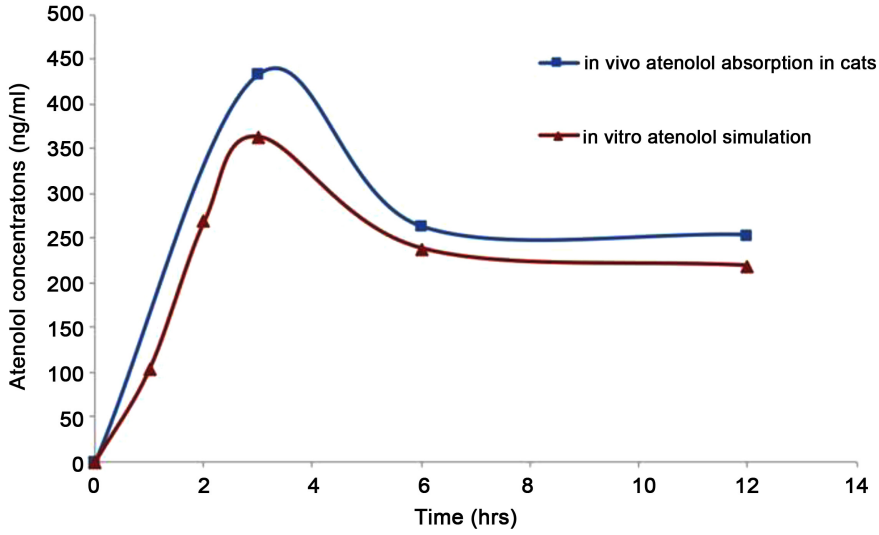


**Figure 5.** Comparison of Actual Atenolol serum concentrations to simulated cat serum concentrations where a burst flux rate of atenolol absorption is followed by a zero-order flux rate of absorption.

in reported in the Quinones paper [6].

## 4. Discussion

Numerous studies have been performed to develop a transdermal drug delivery system for various beta blockers [14]. Though promising none so far has been marketed.

The current study developed a formulation that included stronger percutaneous enhancers that included polysorbate 80, glycerol, propylene glycol, Dimethyl isosorbide (DMI) and ethanol. Carbomer was added at 0.75%. Carbomer content affects atenolol release. Increasing carbomer has been shown to decrease atenolol release [15]. Carbomer content in the current formulation was kept at the lowest level as higher levels of carbomer reduced atenolol release.

The diffusion of atenolol through synthetic membrane is much higher than through cloned epidermis and cat skin. The pore size of the synthetic membrane (0.45 μm) is large in comparison to the molecular size of atenolol. Epidermis skin and cats skin structure are more complicated where drug diffusing through them encounter many other biological and chemical environments than what is encountered in diffusing through the synthetic membrane. Also the epidermis of the cats is a third of the thickness of human epidermis. Cloned human epidermis also has stronger barrier properties than of that found in cat skin. The atenolol diffusion through the cloned human epidermis much lower than among the other barriers tested (synthetic membrane and cat skin).

The results also showed that Formulation 6 is the best in terms of the amount of atenolol that permeated through the human epidermis and cat skin and provided consistent drug release pattern. The individual atenolol fluxes for each formulation indicated that the atenolol proceeds through cloned epidermis and

cat's skin via two fickian flux processes. The first flux is a burst flux which gave the following values: 7.67 to 56.73 (μg/cm$^2$/h) for Formulation 3. For Formulation 5 the Burst Flux 1 ranged from 13.24 to 74.93 (μg/cm$^2$/h) and from 16.516 to 75.07 (μg/cm$^2$/h) for Formulation 6 through cat skin. This is different than what has been reported in atenolol diffusion through porcine and mouse skin [15] [16] which reported release atenolol followed a release pattern to the square root of time. The flux observed followed a single release pattern (no burst effect) for the entire study period with a flux of 65 μg/h/ml.

The second flux is consistent and showed sustained release of atenolol. The second (sustained) Flux 2 ranged from 0.97 to 1.98 (μg/cm$^2$/h) for Formulation 3. The sustained flux for formulation 5 ranged from 1.19 to 2.48 (μg/cm$^2$/h) while that for Formulation 6 has the following range 1.07 to 3.5 (μg/cm$^2$/h) through cat skin.

A simulation of the atenolol cat serum concentrations versus time profile was generated and compared to the actual cat atenolol serum concentration over time. Using a burst effect for the initial absorption of atenolol for the first 3 hours that amounted to 40 percent of the content of atenolol in the dose absorbed followed by a zero-order absorption process that gave an atenolol concentration time curve similar to the actual atenolol cat concentrations time profile. The zero-order absorption rate was 2.7 μg/h. This provided a method to estimate pharmacokinetic parameters for half-life, $K_{el}$, clearance and volume of distribution.

Previous studies have compared atenolol pharmacodynamics after oral and transdermal administration. In healthy male cats receiving oral atenolol compared to transdermal administration reached therapeutic concentrations after oral administration, while cats receiving the transdermal application of atenolol had concentrations below 260 ng/ml at the same time point [7]. Two cats of the seven attained therapeutic atenolol concentrations after transdermal administration with an average peak plasma concentrations for all seven cats of 173 ± 122 ng/ml and mean trough concentrations of 62.4 ± 17 ng/ml. A negative correlation between plasma atenolol concentrations and the cat's heart rate was seen in the transdermal administration cats [7]. The formulation used in the study included glycerol, polysorbate 20, propylene glycol, atenolol and 1% carbomer as gel base [7]. In the current study, the average serum concentration of atenolol in cats initially rose rapidly reaching a peak concentration of 432.7 μg/ml ± 323.3, then rapidly declining to 262.4 μg/ml ± 150.1 at 6 hours and 253.3 μg/ml ± 133.6 at 12 hours. The percentage of cats reaching the therapeutic concentration of 200 μg/ml was 71% at 3 hours, compared to 57 % at 6 hours and 57% at 12 hours. Significantly higher plasma atenolol concentrations were observed in the current study after topical administration by more than two times higher.

A dose of 25 mg per cat every 12 h provided by formulation 6 produced therapeutic serum atenolol concentrations in the majority of cats. The therapeutic atenolol concentration in humans is reported to be 200 μg/ml [17]. In this sce-

nario, all cats reached therapeutic concentrations of atenolol for nearly the entire time. Four cats had a single data point where the serum concentration was not in the therapeutic range (8 data points out of 21 total data points).

The pharmacokinetic model predicted the serum atenolol concentrations. The fit of the model to the serum concentration data versus time confirmed the values of the pharmacokinetic parameters in the Quinone's study (1996). The approach yielded pharmacokinetic parameters of half-life 3.45 h ± 0.5, $K_{el}$ 0.2 $hr^{-1}$ ± 0.3, clearance 260 ml/h/kg ± 72 and volume of distribution 1090 ml/kg ± 148. Therapeutic concentrations at 12 hours post administration after topical atenolol is encouraging. The explanation of the therapeutic atenolol concentrations 12 hours after topical administration is that elimination of the initial burst of drug absorption through the skin has significantly been occurring and the zero-order absorption diffusion process from the second flux of atenolol across the skin membrane has had time to build to where enough atenolol is being absorbed through the skin to sufficiently provide sustained release of therapeutic serum concentrations of atenolol. The atenolol concentration at the 6-hour time point was subtherapeutic most likely due to the intimal burst of topical absorption of atenolol had declined below therapeutic concentrations and the second sustained topical delivery of atenolol had not quite reached therapeutic atenolol concentrations.

The atenolol concentration in the receiver compartment for all formulations in the study was much less than the 1% atenolol applied to the donor compartment. With the extreme differential in atenolol concentrations between the donor and receiver compartments which applied to Fick's law of diffusion readily explains why zero-order release occurs for both fluxes. The atenolol concentration in the donor compartment changes very little so the Fick's equation for diffusion mimics zero-order release.

A drug stability study conducted on the atenolol gel at different temperature conditions (37˚C ± 1˚C and room temperature 25˚C ± 2˚C) for a period of 6 months. No changes (less than 1%) in atenolol concentrations in the formulations were observed after analysis by HPLC.

## 4. Conclusions

Topical administration of atenolol appears to be a viable administration approach to cats. The enhancement that was desired to be obtained on topical administration was reached with formulation 6. A sufficient amount of atenolol diffused through the cat skin produced therapeutic atenolol concentrations. The enhancers that were used in the formulation were sufficient to improve atenolol permeation through cat skin. The enhancers were Polysorbate 80 added at a 5% concentration, Propylene glycol, Glycerol at 15% each to help by increasing skin hydration and are good solvents. Also stronger solvents of Ethanol were added at 10% and Dimethyl-isosorbide (DMI) was added at 15% concentration to be enhancers. The texture of the formulation was adjusted by Carbomer to 0.75%.

Different atenolol formulations were tested and evaluated to get the most effective formulation. The concentration of atenolol in the receiver compartment after 2 hours application to cat skin was 35.5 μg/ml which equates to 497 μg. The therapeutic concentration of atenolol is 263 ng/ml [6] and by considering atenolol has a volume of distribution of ~1000 ml in cat; therapeutic concentrations of atenolol can be attained by topical administration.

The topical administration of atenolol produced therapeutic atenolol levels in cats about 81% of the time. The pharmacokinetic model predicted the serum concentrations using pharmacokinetic parameters from the literature and diffusion fluxes of the study.

Transdermal administration of medications has desired advantages compared to oral and intravenous medications especially in vet medicine. Avoidance of problems of drug administration to certain species should be considered before developing a dosage form, *i.e.* oral administration of cats can be problematic. This study should be a reminder that *in vitro* studies that best predict penetration of drug through skin should be performed using membranes that represent the animal that the test formulation is to be administered to or the drug product is intended to be used in. It is uncertain if these results can be extrapolated to cats with structural heart disease.

## Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this paper.

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## References

[1] Spirito, P., Seidman, C.E., McKenna, W.J. and Maron, B.J. (1997) The Management of Hypertrophic Cardiomyopathy. *The New England Journal of Medicine*, **336**, 775-785. https://doi.org/10.1056/NEJM199703133361107

[2] Schober, K.E., Zientek, J., Li, X., Fuentes, V.L. and Bonagura, J.D. (2013) Effect of Treatment with Atenololon 5-Year Survival in Cats with Preclinical (a Symptomatic) Hypertrophic Cardiomyopathy. *Journal of Veterinary Cardiology*, **15**, 93-104. https://doi.org/10.1016/j.jvc.2013.03.003

[3] Siven, M., Savolainen, S., Rantilila, S., Mannikko, S., Airakisinen, S., Reakallio, M., Vainio, O. and Juppo, A.M. (2016) Difficulties in Administration of Oral Medication Formulations to Pet Cats: An e-Survey of Cat Owners. *Veterinary Record*, **180**, 250. https://doi.org/10.1136/vr.103991

[4] Lecuyer, M., Prini, S., Dunn, M. and Doucet, M.Y. (2006) Clinical Efficacy and Safety of Transdermal Methimazole in the Treatment of Feline Hyperthyroidism. *Canadian Veterinary Journal*, **47**, 131-135.

[5] Sartor, L.L., Trepenanier, L.A., Kroll, M.M., Rodan, J. and Challoner, L. (2004) Efficacy and Safety of Transdermal Methimazole in the Treatment of Cats with Hyperthyroidism. *Journal of Veterinary Internal Medicine*, **18**, 651-655. https://doi.org/10.1111/j.1939-1676.2004.tb02601.x

[6] Quinones, M., Dyer, D.C., Ware, W.A. and Mehvar, R. (1996) Pharmacokinetics of Atenolol in Clinically Normal Cats. *American Journal of Veterinary Research*, **57**, 1050-1053.

[7] Macgregor, J.M., Rush, J.E., Rozanski, E.A., Boothe, D.M., Belmonte, A.A. and Freeman, L.M. (2008) Comparison of Pharmacodynamic Variables Following Oral versus Transdermal Administration of Atenolol to Healthy Cats. *American Journal of Veterinary Research*, **69**, 39-44. https://doi.org/10.2460/ajvr.69.1.39

[8] Kumar, N., Verma, N., Songh, O., Joshi, N. and Singh, K.G. (2010) Estimation of Atenolol by Reverse Phase High Performance Liquid Chromatography. *E-Journal of Chemistry*, **7**, 962-966. https://doi.org/10.1155/2010/601650

[9] Spanakis, M. and Niopas, L. (2010) Determination of Atenolol in Human Plasma by HPLC with Fluorescence Detection: Validation and Application in a Pharmacokinetic Study. *Journal of Chromatographic Science*, **51**, 128-132. https://doi.org/10.1093/chromsci/bms116

[10] Christensen, J.M., Chuong, M., Le, H., Bendas, E. and Pham, L. (2011) Hydrocortisone Diffusion through Synthetic Membrane, Mouse Skin, and EpiDerm™ Cultured Skin. *Archives of Drug Information*, **4**, 10-21. https://doi.org/10.1111/j.1753-5174.2010.00033.x

[11] Khan, G.M., Frum, Y., Sarheed, O., Eccleston and Median, V.M. (2005) Assessment of Drug Permeability Distribution in to Different Model Skins. *International Journal of Pharmaceutics*, **303**, 81-87. https://doi.org/10.1016/j.ijpharm.2005.07.005

[12] Ramteke, K.H., Dighe, P.A., Kharat, A.R. and Patil, S.V. (2014) Mathematical Models of Drug Dissolution: A Review. *Scholars Academic Journal of Pharmacy*, **3**, 388-396.

[13] Nguyen, C.N., Christensen, J.M. and Ayres, J.W. (2008) Formulation of Sustained Release Verapamil HCl and Diltiazem HCl Solid Matrix Capsules. *Pharmaceutical Development and Technology*, **13**, 115-125. https://doi.org/10.1080/10837450701831070

[14] Agil, M., Sultana, Y. and Ali, A. (2006) Transdermal Delivery of Beta Blockers. *Expert Opinion on Drug Delivery*, **3**, 405-418. https://doi.org/10.1517/17425247.3.3.405

[15] Demou, J.S., Sidhom, M.B. and Plakogiannis, F.M. (1994) Comparative *in Vitro* Diffusion Studies for Atenolol Transdermal Delivery System. *Pharmaceutica Acta Helvetiae*, **68**, 215-219. https://doi.org/10.1016/0031-6865(94)90050-7

[16] Gupta, S.P. and Jain, S.K. (2006) Transdermal Atenolol Releasing System: An Approach towards Its Development. *Journal of Drug Targeting*, **14**, 607-613. https://doi.org/10.1080/10611860600866591

[17] Delima, L., Kharasch, E. and Butler, S. (1995) Successful Pharmacological Treatment of Massive Atenolol Overdose: Sequential Hemodynamics and Plasma Atenolol Concentration. *Anesthesiology*, **83**, 204-207. https://doi.org/10.1097/00000542-199507000-00025