

BIOAVAILABILITY OF PROPRANOLOL HCL FOLLOWING ORAL AND TRANSDERMAL ADMINISTRATION IN RABBITS

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تمت صياغة عقار ايدركلوريد البروبرانولول على هيئة هلام ودراسة الامتصاص الجلدي للعقار من هذه الصيغة ومقارنتها بامتصاصه عقب تناول محلوله الفموي وذلك باستخدام الارانب. وقد استخدمت طريقة كروماتوجرافيا السوائل ذات الكفاءة العاليه في تعيين تركيز العقار في البلازما. وقد نتج عن استخدام الهلام الجلدي مستويات ممتده ومقبوله من العقار في الدم. وقد انعكس هذا في الزيادة الكبيره في معاملات متوسط زمن بقاء العقار في الجسم ومتوسط زمن امتصاصه الخاصه بالهلام مقارنة بالمعاملات الخاصه بالمحلول الفموي. وقد وجد أن متوسط نسبة الاتاحة الحيويه المطلقة للهلام ٥٣٪ مقارنة ب ٨,٨٪ للمحلول الفموي. وفسرت هذه النتائج على أنها تمثل - في الغالب - انعكاسا للامتصاص المستمر للعقار من الهلام الجلدي بالاضافة الى الايض الكبدي الكبير للعقار عند مروره لأول مره عقب امتصاصه من المحلول الفموي.

Propranolol hydrochloride gel formulation was developed and the in-vivo percutaneous absorption of the drug was studied and compared with oral absorption using rabbits as animal model. Drug plasma concentrations were determined using a validated HPLC method of assay. The gel formulation appeared to produce acceptable sustained blood levels of the drug and the mean residence time (MRT) and the mean absorption time (MAT) were dramatically increased following gel application as compared to the oral solution administration. The mean absolute bioavailability (F%) was found to be 8.8% for oral administration and 53% for the transdermal formulation. These results could be, at least in part, due to the continuous absorption of drug from gel formulation as well as the substantial hepatic first-pass metabolism associated with the oral administration of the drug.

INTRODUCTION

Propranolol hydrochloride (PL) is a non-selective β -adrenergic blocker and widely used in the treatment of angina, hypertension, and in decreasing mortality in patients suffering from myocardial infarction.¹

Propranolol hydrochloride is almost completely absorbed from the gastrointestinal tract, but is subject to considerable hepatic tissue binding and first-pass metabolism,² with reported systemic bioavailability of between 15 and 23%.³ Delivery of PL via the rectal⁴ and vaginal⁵ routes has avoided some of the first-pass metabolism associated with the oral administration, achieving bioavailability of between 40-50%.

The transdermal route of administration has been demonstrated to be capable of avoiding the hepatic first-pass effect, thus achieving higher systemic bioavailability of drugs.⁶ Propranolol has a low molecular weight, high lipid solubility as a base, and effective low plasma concentration.⁷ These characteristics could make PL a good candidate to be administered transdermally.

Previous work in our laboratory has demonstrated that propranolol HCl (PL) was effectively absorbed percutaneously. Different PL gel formulations were prepared using different polymers and enhancers. The *in-vitro* percutaneous absorption of the drug through rabbit skin indicated that the highest rate and the greatest extent of absorption was obtained from

hydroxypropyl methylcellulose gel formulation containing 9% w/w 1,8-cineol as enhancer⁸

The objective of the present work was to evaluate the systemic bioavailability of PL in rabbits following the transdermal application of the developed gel formulation and compare it with that of oral solution using crossover study. In this regard, the intravenous administration of drug was included in order to determine the absolute bioavailability (F%) and the mean absorption time (MAT) for each of the oral and transdermal dosage form.

EXPERIMENTAL

Materials

Propranolol HCl (Arab Pharmaceutical Manufacturing Co., Ltd., Amman, Jordan), Indenolol HCl (Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan), Hydroxypropyl methylcellulose (Sigma Chem. Co., St. Louis, MO., USA), 1,8-Cineol (BDH Chemicals Ltd., Poole, UK). All other chemicals were analytical grade and solvents were HPLC grade.

Preparation of gel

A weighed amount of 1.5 g of hydroxypropyl methylcellulose was dispersed in an aliquot of hot water containing 0.037 g of methyl paraben. The dispersion was cooled in ice to form the gel. A volume of 10 ml of cold propranolol aqueous solution (20 mg/ml) containing sodium metabisulphite (0.025 g) was added, slowly, to the gel with continuous stirring. Similarly, a weighed amount of 2.25 g of 1,8 cineol - the enhancer - was then added. Care was taken to avoid the incorporation of air bubbles. The pH of the gel was adjusted to 6.5 by adding few drops of 1 N sodium hydroxide. The final weight of the preparation was adjusted to 25 g by the addition of water.

Preparation of propranolol injection

An appropriate amount of propranolol was dissolved in sterile normal saline for injection in laminar air flow cabinet to prepare 1 mg/ml solution. The obtained solution was filtered using bacterial filter (Milex-GS, 0.22 µm bacterial filter, Millipore Corp., Bradford, MA, USA). The solution was prepared and used immediately.

Animals

Ten white New Zealand male rabbits weighing 3.6-4.4 kg were utilized in the study. The animals were kept on rabbit chow and housed individually in an animal room maintained at 25°. The animals were fasted 12 hours before and during experiment and water was allowed *ad libitum*. Prior to the administration of the drug, hair was removed from the ears of the rabbits using an animal clipper.

Study design and plasma samples:

Propranolol was administered to all animals on three occasions separated by 3-week washout period after each treatment. All animals were received a dose of 1 mg/kg propranolol solution as an i.v. injection through the right ear marginal vein. The injection time was less than one minute. Blood samples, 1.5 ml each, were collected in 5 ml heparinized evacuated blood collection tubes from the left ear marginal vein by using a catheter (18 G X 1¼ inch) at the following time intervals: 0, 10, 20, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240, 300, and 360 minutes post injection.

In the second stage of the study, rabbits were randomly divided into 2 groups and assigned to 1 of the 2 sequences of drug administration. In this stage, the study design was 2-treatment, 2-period, 2-sequence crossover study. Each rabbit was received the drug in 2 occasions. Once as an oral solution and in the second occasion as a transdermal gel.

In oral administration, each animal was received 15 ml drug solution (2 mg/ml) in distilled water via gastric tube as a bolus, followed by 10 ml of distilled water to flush the tube. Blood samples were collected just prior to drug administration and at 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, and 480 minutes post drug administration.

One day before transdermal application of the gel, the hair was removed from an area of (5 cm x 5 cm) of the back of each rabbit using an animal electric hair clipper. Care was taken not to damage the skin. The skin was examined under a high-powered magnifying glass to make sure that no damage was resulted from the shaving process. The rabbits were immobilized in a restraining rack during the entire experiment to prevent them from removing the

applied gel. A circle with 2 cm diameter (area = 3.14 cm²) was marked with a marker pen on the shaved skin of the rabbit. A dose of 20 mg of PL was spread uniformly over the designated area. Blood samples, 1.5 ml each, were collected from the right ear marginal vein at the following time intervals: 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 24.0 hrs post application.

In all cases, blood samples were centrifuged immediately for 4 minutes at 4000 rpm, and plasma samples were transferred to clean 4 ml polypropylene tubes and stored at -20° pending analysis.

Analysis of plasma samples

Concentration of PL in plasma samples were determined using HPLC, with indenolol HCl as internal standard.⁹

Pharmacokinetic analysis

The plasma concentrations following intravenous administration of the drug were analyzed by a linear two-compartment open pharmacokinetic model with elimination from the central compartment. The plasma concentration of PL (C_p) as a function of time (t) was described by the following equation:¹⁰

$$C_p = A e^{-\alpha t} + B e^{-\beta t}$$

where A, B, α, and β denote hybrid constants for the distribution and elimination phases, respectively. The relevant pharmacokinetic parameters such as the distribution half-life (t_{1/2α}), the terminal elimination half-life (t_{1/2β}), the area under the plasma concentration-time curve (AUC_{0-∞}), the area under the first moment curve (AUMC_{0-∞}), and the mean residence time of the drug in the body (MRT) were calculated using the following equations:¹⁰

$$t_{1/2\alpha} = 0.693 / \alpha$$

$$t_{1/2\beta} = 0.693 / \beta$$

$$AUC_{0-\infty} = A/\alpha + B/\beta$$

$$AUMC_{0-\infty} = A/\alpha^2 + B/\beta^2$$

$$MRT = AUMC_{0-\infty} / AUC_{0-\infty}$$

Pharmacokinetic parameters for propranolol following oral and transdermal administration were determined from the plasma concentration-time data. The maximum plasma concentration (C_{max}) and its corresponding time

(T_{max}) were obtained directly from the individual plasma concentration-time data. The area under the plasma concentration-time curve up to the last measurable plasma concentration (AUC_{0-t}) and the area under the first moment curve (AUMC_{0-t}) were estimated by linear trapezoidal rule and extrapolated to infinity using the following equations:¹⁰

$$AUC_{0-\infty} = AUC_{0-t} + C_{pt} / K_{el}$$

$$AUMC_{0-\infty} = AUMC_{0-t} + t.C_{pt} / K_{el}$$

where:

C_{pt} is the last measurable concentration at time t.

K_{el} is the terminal elimination rate constant calculated by least-square regression analysis.

The elimination half-life (t_{1/2}) was calculated as:

$$t_{1/2} = 0.693 / K_{el}$$

The mean residence time of the drug in the body (MRT) was calculated using the following equation:¹¹

$$MRT = AUMC_{0-\infty} / AUC_{0-\infty}$$

The mean absorption time (MAT) and the absolute bioavailability (F) for the oral solution and transdermal gel formulation were calculated using the following equations:¹²

For oral solution:

$$MAT = MRT_{po} - MRT_{i.v.}$$

$$F = AUC_{po} / AUC_{i.v.} \times Dose_{i.v.} / Dose_{po}$$

And for transdermal (TS) administration:

$$MAT = MRT_{TS} - MRT_{i.v.}$$

$$F = AUC_{TS} / AUC_{i.v.} \times Dose_{i.v.} / Dose_{TS}$$

where MRT_{i.v.}, MRT_{po}, and MRT_{TS} are the mean residence time after i.v., oral, and transdermal administration, respectively.

Statistical analysis

The pharmacokinetic parameters of PL calculated following oral and transdermal administrations were evaluated statistically using two-way ANOVA. Differences between two related parameters were considered statistically significant for p ≤ 0.05.

RESULTS AND DISCUSSION

The disposition of propranolol in rabbits following a bolus intravenous administration of 1 mg/kg dose was adequately described by a two-compartment open model with first order rates. The concentration of PL in plasma can be described by the following equation:

$$C_p = 124.5 e^{-3.22t} + 74.2 e^{-0.45t}$$

The mean apparent half-life of the α -phase was found to be 0.23 hrs and the mean half-life of the β -phase was 1.56 hrs. The reported values for the half-life of the elimination phase following intravenous administration of PL in rabbits were 1.44 and 1.74 hrs, respectively.^{13,14} The values for the pharmacokinetic parameters of PL following intravenous administration are listed in Table (1) and the mean plasma concentration-time profile is depicted in Figure (1).

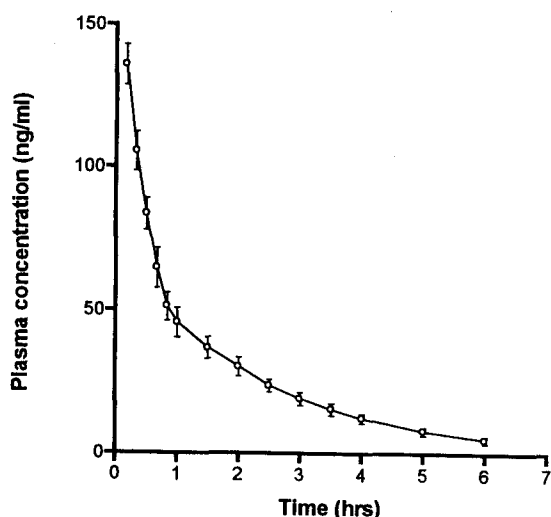


Fig. 1: Mean \pm SD Plasma Concentration of Propranolol (ng/ml) after Intravenous Administration of 1 mg/kg Dose to Ten Rabbits.

The absorption of PL, following oral administration of 30 mg dose, was rapid (Figure 2). The mean peak plasma concentrations of 68.8 ng/ml was observed after 0.25 hrs following the dose. The plasma levels of PL decreased to a mean concentration value of 4.5 ng/ml after 6 hrs. Thus, it is evident that a high blood level of PL was achieved with oral administration.

After applying the transdermal gel containing 20 mg dose of the drug, the mean peak plasma concentrations of PL was 39 ng/ml that achieved at a mean time of 1.9 hrs post application. After that, the plasma levels of PL decreased slowly to a mean value of 6.8 ng/ml at 24 hrs sampling time (Figure 2).

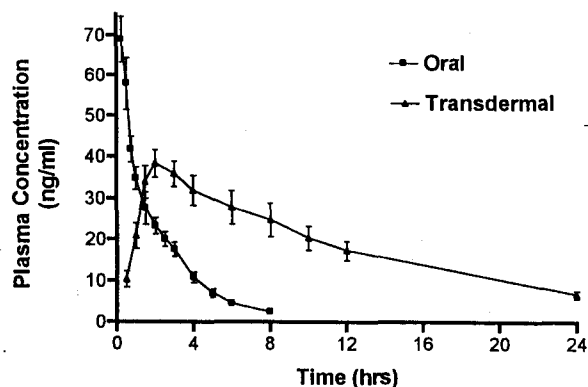


Fig. 2: Mean \pm SD Plasma Concentration of Propranolol (ng/ml) after Oral Administration (30 mg Dose) and Transdermal Application (20 mg Dose) to Ten Rabbits.

The gel formulation appeared to produce acceptable sustained blood levels of PL without producing the high initial blood levels seen for the oral solution dosage form. In addition, PL was measurable at the last sampling time (24 hrs) in all rabbits following transdermal administration of the gel formulation, whereas, the drug was not measurable after the last sampling period (8 hrs) after administration of the oral solution.

The sustained release characteristics of the transdermal gel formulation were also reflected in the mean residence time (MRT) and the mean absorption time (MAT) of propranolol in the body. Both time parameters were dramatically increased following transdermal gel administration compared to the oral solution administration. The mean MRT values for the transdermal gel formulation and oral solution were 13.38 and 2.39 hrs, respectively, whereas, the mean MAT values were 11.5 and 0.51 hrs, for transdermal and oral solution, respectively (Table 2).

Table 1: Pharmacokinetic Parameters* Following I.V. Administration of 1 mg/kg Propranolol.

Parameter	Value \pm SD
α (hr ⁻¹)	3.22 \pm 0.69
β (hr ⁻¹)	0.45 \pm 0.05
A (ng/ml)	124.4 \pm 18.5
B (ng/ml)	74.2 \pm 9.2
AUC _{0$\rightarrow$$\infty$} (ng.hr.ml ⁻¹)	205.3 \pm 15.8
AUMC _{0$\rightarrow$$\infty$} (ng.hr ² .ml ⁻¹)	387.4 \pm 63.4
MRT (hr)	1.88 \pm 0.19

*Parameters are given \pm standard deviation.

Table 2: Pharmacokinetic Parameters* of Propranolol Following Oral (30 mg dose) and Transdermal (20 mg dose) Administration to Ten Rabbits.

Parameter	Oral	Transdermal
AUC _{0$\rightarrow$$\infty$} (ng.hr.ml ⁻¹)	134.6 \pm 10.9	540.3 \pm 48.6
C _{max} (ng ml ⁻¹)	68.8 \pm 5.7	39.6 \pm 3.2
T _{max} (hr)	0.25	1.9 \pm 0.2
MRT (hr)	2.39 \pm 0.2	13.38 \pm 1.1
MAT (hr)	0.51 \pm 0.25	11.5 \pm 1.1
F %	8.8 \pm 1.1	52.9 \pm 7.7

*Parameters are given \pm standard deviation.

The results obtained indicated an increase in the half-life of PL in rabbits following transdermal administration. The mean value obtained (8.94 hrs) was found to be longer than the mean values obtained following intravenous and oral administration; 1.56 and 1.58 hrs, respectively. The long half-life observed in the case of transdermal application is probably due to continued absorption of the drug from the transdermal formulation. In other words, the obtained half-life (8.94 hrs) is not the true elimination half-life but a composite of an apparent absorption and elimination. Similar long half-life was observed for long acting propranolol (15,16). This might explain the dramatic increase in the mean residence and

mean absorption times following the transdermal administration. The increase in the area under the plasma propranolol versus time curve after the application of the gel might also be a consequence of the prolonged elimination half-life of propranolol.

Analysis of variance of the pharmacokinetic parameters (AUC_{0 \rightarrow ∞} , C_{max}, T_{max}, MRT, and MAT) clearly indicated statistically significant differences ($p \leq 0.05$) between the transdermal and oral route of administration in all pharmacokinetic parameters.

The mean absolute bioavailability (F%) was determined and found to be 8.78% for the oral administration and 52.96% for transdermal

formulation. As expected, oral administration of PL resulted in a low bioavailability due to the extensive hepatic first-pass metabolism. The higher bioavailability of the gel formulation compared to the oral solution can be explained, at least in part, to the continuous drug absorption from this formulation for a longer period of time and due to that transdermal delivery can avoid a substantial amount of the hepatic first-pass metabolism associated with the oral route.

In conclusion, the data generated from this study provided useful information about the absorption characteristics of PL transdermal gel formulation. This study revealed that, such formulation produced sustained absorption of the drug with an increase in the extent of absorption and longer residence time of the drug in the body without the risk of dose dumping seen with the oral administration.

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