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Research paper

Comparative in vivo evaluation of propranolol hydrochloride after oral and transdermal administration in rabbits

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Abstract

The purpose of this study was the in vivo evaluation of orally and transdermally administered propranolol hydrochloride in rabbits. Transdermal patches of propranolol hydrochloride (PPN) were formulated employing ethyl cellulose and polyvinylpyrrolidone as film formers. The pharmacodynamic (PD) and pharmacokinetic (PK) performance of PPN following transdermal administration was compared with that of oral administration. This study was carried out in a randomized cross-over design in male New Zealand albino rabbits. The PK parameters such as maximum plasma concentration (C_{max}), time for peak plasma concentration (t_{max}), mean residence time (MRT) and area under the curve ($AUC_{0-\alpha}$) were significantly ($P < 0.01$) different following transdermal administration compared to oral administration. The terminal elimination half-life ($t_{1/2}$) of transdermally delivered PPN was found to be similar to that following oral administration. In contrast to oral delivery, a sustained therapeutic activity was observed over a period of 24 h after transdermal administration compared to oral administration. The relative bioavailability of PPN was increased about fivefold to sixfold after transdermal administration as compared to oral delivery. This may be due to the avoidance of first pass effect of PPN. The sustained therapeutic activity was due to the controlled release of drug into systemic circulation following transdermal administration.

Keywords: Transdermal delivery; Propranolol hydrochloride; Pharmacodynamics; Pharmacokinetics

1. Introduction

Propranolol hydrochloride is a β-blocker widely used in the treatment of angina pectoris, cardiac arrhythmias and hypertension [1]. It is subjected to extensive and highly variable hepatic first pass metabolism following oral administration, with a reported systemic bioavailability between 15 and 23% [2]. Prolonged release formulations may reduce the dosing frequency [3-5] but the bioavailability of PPN from these formulations is only 40–60% of that from a conventional tablet. This has been attributed to the slower absorption in the gastrointestinal tract coupled with an extensive first pass effect [6,7]. Hence, in the present study, the in vivo performance of orally and transdermally administered PPN was evaluated in rabbits in a randomized cross-over design.

The polymeric films containing ethyl cellulose:polyvinylpyrrolidone (PVP):drug (9:1:3, 8:2:2 and 8:2:3) were selected for transdermal administration based on in vitro studies [8]. The polymeric films were prepared by the mercury substrate method employing dibutyl phthalate as plasticizer. The dried polymeric films were evaluated using different parameters including thickness uniformity, drug content of the film, in vitro drug release from films and in vitro skin permeation of drug, prior to their in vivo evaluation.

2. Materials and methods

2.1. Materials

Propranolol hydrochloride (gift sample from M/s. Natco Pharma Ltd., Hyderabad, India), urethane–isoprenaline (Sigma Chemicals Co., St. Louis, MO), ethyl cellulose (14 cps), PVP (Mol. wt. 40,000), dibutyl phthalate, sodium

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hydroxide, hydrochloric acid and sodium chloride were of pharmaceutical grade and chloroform was of HPLC grade and obtained commercially.

2.2. Animals

New Zealand male rabbits (1.2–1.8 kg) were obtained from National institute of Nutrition, Hyderabad, India, and maintained at 25 ± 1°C for the study. The animals were housed in stainless steel metabolic cages and provided with standard diet and water ad libitum. Necessary approvals were obtained for conducting this study.

2.3. Preparation of polymeric films

The polymeric films composed of ethyl cellulose:PVP: drug (9:1:3, 8:2:2 and 8:2:3) were prepared by mercury substrate method [9]. Dibutyl phthalate was incorporated, at a concentration of 30% w/w of dry weight of polymers, as plasticizer. Briefly, the method involved the pouring of a chloroform solution containing drug, polymers and plasticizer on a mercury surface contained in a petri dish. The rate of evaporation of the solvent was controlled by placing an inverted funnel over the petri dish. The dry films were removed from the mercury surface and kept in a desiccator until use.

The thickness of dried films was measured at five different places using a micrometer (MITUTOYO, Japan) and the mean values were calculated. The uniformity of drug content of the films was determined, based on the dry weight ratios of drug and polymers used by means of a UV spectrophotometric method. A thin layer chromatographic method was used to follow the drug–carrier interactions. Paddle over disk method was employed for the in vitro drug release determination from the films. The in vitro skin permeation of drug from the films was studied by using modified Franz diffusion cells fastened with 'O' ring.

2.4. Pharmacokinetic studies

The pharmacokinetic (PK) performance of PPN following oral and transdermal administration was studied in a randomized cross-over design in rabbits. Animals were fasted 24 h prior to the administration of drug formulations but had free access to water. One day prior to the experiment, hair on the abdominal area was clipped by applying a depilatory agent for 10 min and washed with distilled water. On the day of experiment, the animals were anaesthetized with urethane (1 g/kg, i.p.). Following anaesthesia, animals were secured in a supine position. The polymeric film (18 cm^2) was applied on hair free abdominal skin with a pressure sensitive adhesive and occluded with plaster. Drug solution was administered orally (10 mg/kg) with a soft plastic tube. Blood samples (~1.5 ml) were collected at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 and 6.0 h after oral and 0, 2.0, 4.0, 8.0, 12.0, 20.0, 24.0, 26.0, 28.0 and 30.0 h after transdermal administration from orbital sinus into a heparinized glass tubes. The plasma was separated immediately and frozen at −20°C until analysis.

2.5. Estimation of propranolol in plasma

Plasma levels of propranolol were estimated by modified spectrofluorometric method [10]. To 0.5 ml plasma, 0.1 ml of 0.01 N HCl was added and then alkalinized with 0.2 ml of 2 N NaOH and extracted with 6 ml of chloroform for 15 min. Organic layer (5 ml) was separated and evaporated to dryness at 40°C under a stream of nitrogen. The residue was reconstituted with 3 ml of 0.01 N HCl and then propranolol was estimated using Spex Fluorolog Spectrofluorimeter with 290 and 360 nm as excitation and emission wavelength, respectively. The lower detection limit by this method was 5 ng/ml. The coefficients of variation for intra- and inter-day estimations were 1.4 and 1.6, respectively, demonstrating good reproducibility.

2.6. Pharmacokinetic analysis

PK parameters such as peak plasma concentration (C_{max}) and time of its occurrence (t_{max}) were read directly from the individual plasma concentration–time profiles. The other PK parameters, e.g. biological half-life ($t_{1/2}$), mean residence time (MRT) and area under the curve ($AUC_{0-\alpha}$), were calculated using a computer programme 'RAMKIN' which measures $t_{1/2}$ from the regression of the terminal phase of concentration time plot. MRT was calculated by dividing the $AUMC_{0-\alpha}$ by $AUC_{0-\alpha}$ and $AUC_{0-\alpha}$ was calculated by linear trapezoidal rule. The differences in various PK parameters were evaluated statistically by ANOVA.

2.7. Pharmacodynamic studies

Pharmacodynamic (PD) performance in animals following oral and transdermal administration of PPN was assessed by the percent β-blockade. The animals were fasted for 24 h prior to the administration of drug formulations but had free access to water. Animals were anaesthetized with urethane (1 g/kg, i.p.). Following anaesthesia normal electrocardiogram (ECG) for all the animals was recorded (Lead II, Eta cardiograph, UGO Basile, Italy). Before administering the drug formulations, isoprenaline (2 μg/kg, i.v.) in normal saline was administered into the marginal ear vein. ECG was recorded immediately and these cardiograms served as control. Animals were left for one hour and the drug formulations were administered as described in PK studies. ECG was recorded after 1, 2, 4 and 6 h in case of oral and 4, 8, 12, 20 and 24 h in case of transdermal post-administration, isoprenaline (2 μg/kg, i.v.) response was recorded as before. Each animal was used as its own control. The percent β-blockade was determined by taking the difference in the response of isoprenaline at 0 time

Table 1
In vitro cumulative % release of propranolol HCl from various polymeric films of EC:PVP:drug (Mean ± SD; $n = 3$)

Time (h)	Cumulative % drug released		
	9:1:3 (45[a])	8:2:2 (30[a])	8:2:3 (45[a])
0.0	0.00	0.00	0.00
0.5	17.75 ± 0.477	24.80 ± 0.344	30.27 ± 0.666
1.0	22.68 ± 0.522	34.05 ± 0.416	33.38 ± 0.694
1.5	26.63 ± 0.661	38.91 ± 0.483	39.38 ± 0.800
2.0	30.18 ± 0.688	44.73 ± 0.716	42.92 ± 0.833
2.5	32.92 ± 0.766	48.84 ± 1.477	47.20 ± 0.783
3.0	35.55 ± 0.078	52.88 ± 0.583	51.15 ± 0.988
3.5	38.35 ± 0.877	56.81 ± 0.544	54.54 ± 0.788
4.0	40.45 ± 0.091	60.41 ± 0.544	57.70 ± 0.922
4.5	42.72 ± 0.877	63.42 ± 0.594	60.46 ± 0.977
5.0	44.59 ± 1.050	66.01 ± 0.600	63.14 ± 0.922
6.0	48.48 ± 0.855	72.36 ± 0.566	68.26 ± 1.061
7.0	51.94 ± 0.861	77.94 ± 0.661	73.48 ± 1.050
8.0	55.56 ± 1.255	82.05 ± 1.016	78.04 ± 0.911
9.0	58.18 ± 1.227	87.02 ± 0.866	80.48 ± 1.083
10.0	61.36 ± 1.022	91.80 ± 0.883	82.24 ± 1.177

[a] Amount of drug (mg) present in 18 cm^2 of the film.

and at the specified time intervals post-administration of drug formulations.

3. Results

The polymeric films were smooth, uniform and flexible. Preliminary studies revealed that the drug was uniformly distributed throughout the film and that there were no interactions between the drug and carriers used. Both in vitro drug release and skin permeation increased with increasing drug loading and PVP content (Tables 1 and 2 and Figs. 1 and 2). The optimum flux was obtained using the following ratios of ethyl cellulose:PVP:drug, 9:1:3, 8:2:2 and 8:2:3 (Table 3). Hence, these were selected for the in vivo experiments.

Table 2
In vitro skin permeation of propranolol HCl from various polymeric films of EC:PVP:drug (Mean ± SD; $n = 3$)

Time (h)	Cumulative amount of drug permeated (μg)		
	9:1:3 (11.25[a])	8:2:2 (7.5[a])	8:2:3 (11.25[a])
0	0.00	0.00	0.00
1	019.33 ± 3.51	041.67 ± 3.79	046.33 ± 6.51
2	058.33 ± 6.03	105.33 ± 3.06	121.33 ± 12.66
3	145.67 ± 6.51	197.67 ± 6.66	234.67 ± 20.13
4	215.00 ± 9.00	275.67 ± 8.02	316.33 ± 27.19
5	287.00 ± 6.56	350.33 ± 10.69	401.67 ± 19.76
6	358.33 ± 9.71	431.33 ± 15.02	502.67 ± 28.54
7	440.67 ± 9.61	516.33 ± 14.50	585.33 ± 24.50
8	513.00 ± 13.45	601.33 ± 16.44	662.33 ± 23.50
9	580.00 ± 12.53	662.33 ± 13.05	752.00 ± 39.15
10	655.33 ± 14.57	749.67 ± 14.50	823.67 ± 27.50
11	735.33 ± 12.06	844.33 ± 19.60	921.33 ± 41.49
12	817.00 ± 16.09	905.33 ± 24.01	995.67 ± 30.66

[a] Amount of drug (mg) present in 4.5 cm^2 of the film.

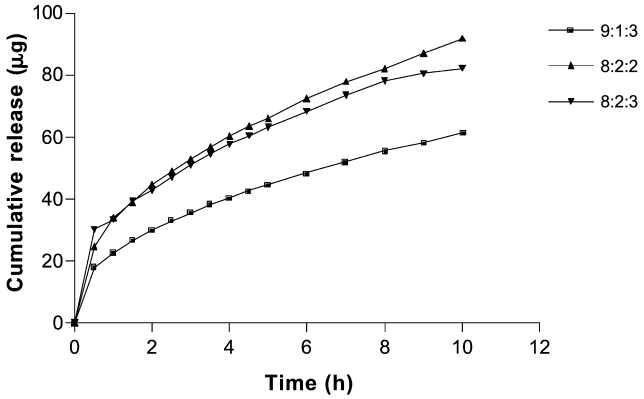

Fig. 1. In vitro cumulative % drug release–time profiles of EC:PVP:drug at different concentrations.

The mean plasma concentration–time profiles of PPN after oral and transdermal administration are shown in Figs. 3 and 4, respectively. The calculated PK parameters are given in Table 4. The results from the oral administration of PPN indicated that it was rapidly absorbed from the rabbit gastrointestinal tract with a C_{max} of 111.8 ± 16.5 ng/ml at a t_{max} of 0.42 ± 0.13 h. Transdermal administration of PPN achieved steady state plasma concentration of 62.7 ± 14.2, 76.7 ± 8.3 and 77.0 ± 8.2 ng/ml after an initial lag time of approximately 10–12 h. Upon removal of the transdermal device, a mild reservoir effect was observed for about 2 h followed by normal elimination similar to that after oral administration.

The oral administration of PPN resulted in an $AUC_{0-\alpha}$ of 328.9 ± 81.0 ng/ml/h, whereas, the transdermal administration resulted in a bioavailability of 1433.2 ± 346.7–1850.5 ± 215.2 ng/ml/h. Similarly, the MRT after transdermal administration was higher (16.1 ± 0.4–16.5 ± 0.2 h) compared to oral administration

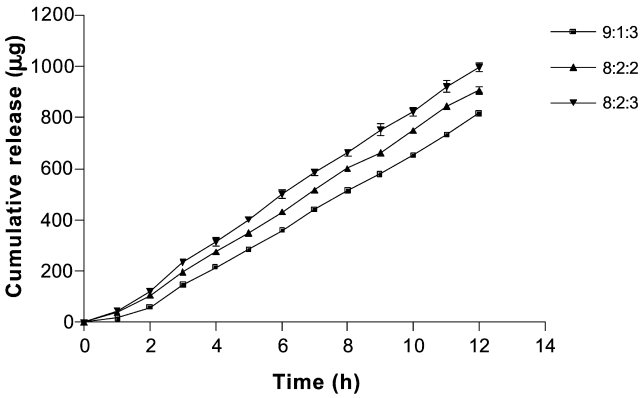

Fig. 2. In vitro skin cumulative drug release–time profiles of EC:PVP:drug at different concentrations.

Table 3
Drug content, Rf, in vitro release rate and skin flux of propranolol HCl from various polymeric films (Mean ± SD; $n = 3$)

Film composition (EC:PVP:Drug)	Percent drug content	Rf value	Release rate constant (mg/cm^2.min$^{1/2}$)	Skin flux (μg/cm^2.h)
9:1:3	99.4 ± 1.8	0.81	0.041 ± 0.006	14.29 ± 0.083
8:2:2	101.6 ± 2.5	0.84	0.042 ± 0.008	15.83 ± 1.07
8:2:3	98.4 ± 1.6	0.82	0.057 ± 0.012	17.16 ± 1.34

Table 4
Pharmacokinetic parameters obtained after oral an transdermal administration of propranolol hydrochloride in rabbits (Mean ± SD; $n = 6$)

Parameter	Oral	Transdermal (EC:PVP:Drug)		
		9:1:3	8:2:2	8:2:3
C_{max} (ng/ml)	111.8 ± 16.5	62.7 ± 14.2	76.7 ± 8.3	77.0 ± 8.2
t_{max} (h)	0.42 ± 0.13	10.0 ± 2.2	12.0 ± 4.4	10.7 ± 2.1
$t_{1/2}$ (h)	1.68 ± 0.2	2.0 ± 0.2	2.0 ± 0.2	2.1 ± 0.3
MRT (h)	2.82 ± 0.2	16.1 ± 0.4	16.5 ± 0.2	16.3 ± 0.2
$AUC_{0-\alpha}$ (ng/ml/h)	328.9 ± 81.0	1433.2 ± 346.7	1788.0 ± 246.5	1850.5 ± 215.2

(2.82 ± 0.2 h). The units of bioavailability are concentration x time; therefore, ng h/ml or ng h ml^{-1}.

The percent β-blockade following oral and transdermal administration is given in Table 5. In agreement with PK data, maximum β-blockade was obtained at 1 h after oral administration and decreased by 80% after 6 h. In the case of transdermal administration, a steady state β-blockade was observed after 12.0 h and was prolonged over a period of 24 h. Further, it was observed that there are no significant changes on skin surface after the removal of polymeric films.

4. Discussion

Transdermal delivery offers several advantages over oral routes for controlled drug delivery [11] viz., avoidance of hepatic first pass metabolism, the ability to control drug delivery for a longer time than the gastrointestinal transit of oral dosage forms, the ability to avoid a changing physiological environment and chemical or metabolic degradation, the ability to discontinue administration by removal of the system. The dermally applied materials, absorbed in quantities large enough to elicit a pharmacological effect, has been known for years [12].

The burst effect at elevated drug and/or PVP content was due to the rapid dissolution of the surface drug. The rapid leaching out of PVP results in the formation of pores and thus lead to the decrease of mean diffusional path length of the drug molecules to release into dissolution medium and hence, higher release rates.

The enhancement of skin flux with increase of drug concentration may be due to accumulation of high amounts of drug on the skin surface. The improvement of skin flux with increase of PVP may be attributed to its anti-nucleating

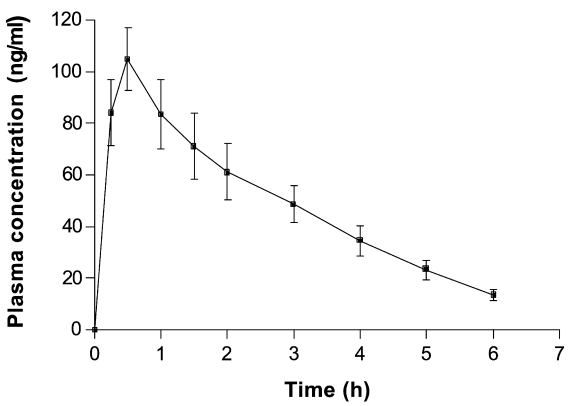

Fig. 3. Plasma concentration–time profile of propranolol hydrochloride after oral administration in rabbits ($n = 6$).

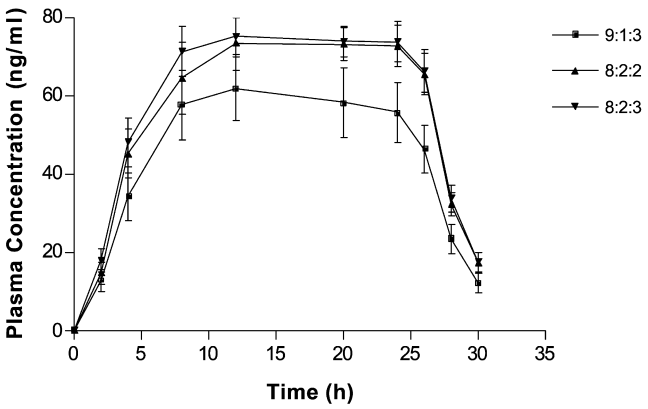

Fig. 4. Plasma concentration–time profiles of propranolol hydrochloride after transdermal administration of polymeric films containing different ratios of EC:PVP:drug, in rabbits ($n = 6$).

Table 5
Percent β-blockade after oral and transdermal administration of propranolol HCl in rabbits (Mean ± SD; $n = 6$)

Formulation	Percent β-blockade							
	1 h	2 h	4 h	6 h	8 h	12 h	20 h	24 h
Oral	100.2 ± 11.7	80.1 ± 12.9	52.4 ± 11.9	20.5 ± 7.1				
Transdermal EC:PVP:drug								
9:1:3			50.7 ± 10.3		78.0 ± 9.6	84.6 ± 8.7	80.4 ± 13.0	83.8 ± 15.1
8:2:2			58.5 ± 18.8		80.0 ± 24.1	91.9 ± 19.2	93.0 ± 14.1	96.8 ± 13.9
8:2:3			60.6 ± 15.8		90.6 ± 13.7	95.1 ± 11.8	97.9 ± 10.4	96.7 ± 10.4

effect that converted the crystalline drug into amorphous state, which generally possesses a high-energy state with improved solubility. The enhancement of solubility of drug increases thermodynamic activity that facilitates the permeation rate of drug through the skin.

The low t_{max} and high C_{max} values following oral administration were due to rapid absorption from the gastrointestinal tract. In contrast, the low C_{max} and prolonged t_{max} after transdermal administration of polymeric films were due to the barrier properties of the skin which lead to an early accumulation of the drug in the skin followed by a sustained release into the systemic circulation. The summary of the $AUC_{0-\alpha}$ of propranolol after oral and transdermal administration revealed a fivefold to sixfold increase in the bioavailability following transdermal delivery as compared to oral administration. The increase in bioavailability following transdermal administration is due to avoidance of substantial amount of hepatic first pass metabolism associated with oral administration. The reservoir effect after removal of the polymeric films might be due to the slow depletion of the drug accumulated in skin tissues. The observation that the $t_{1/2}$ values were indicated after oral and transdermal administration was due to the fact that the elimination of drug follows first order kinetics and hence, the terminal $t_{1/2}$ are similar. The higher MRT values following transdermal delivery compared to the oral route may be due to continuous replenishment of drug into the systemic circulation by constant and controlled delivery of drug from the transdermal patch.

The low β-blockade activity after oral administration is in good agreement with PK data, which indicates that the plasma concentration of propranolol declined rapidly and hence, the shorter duration of therapeutic action. The sustained β-blockade response following transdermal administration was due to controlled and continuous release of drug into the systemic circulation over an extended period of study.

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