Labetalol Absorption Kinetics: Rat Small Intestine and Colon Studies

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ABSTRACT: Labetalol is a widely used drug for the management of hypertension, which is preferably administered by the oral route despite its low bioavailability. The objective of this study is to ascertain the mechanisms underlying its absorption as an approach to help in predicting the influence of dosage changes, possible drug–drug and drug–fruit juice interactions. Perfusion experiments have been performed in rats in two sites of absorption: the intestine and the colon. The nonlinearity of the process has been established by means of the assay of a wide range of concentrations (2–2000 μM). Fitting of the concentration versus time data allows the estimation of passive diffusion constant in the intestine (1.42 ± 0.05/h) and the colon (1.13 ± 0.06/h), V_m and K_m of the input process (9.85 ± 4.98 μM/h, and 10.44 ± 26.16 μM, respectively) and K_m of an efflux system (0.53 ± 1.16 μM) and V_m in both intestinal segments (2.60 ± 11.37 μM · /h in the intestine and 0.66 ± 1.38 μM · /h in the colon). The efflux carrier implicated is identified by means of several inhibition experiments, whose inhibition ability is mathematically estimated. Results suggest the p-glycoprotein as responsible for the efflux of labetalol. J Pharm Sci 95:1733–1741, 2006

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INDRODUCTION

Labetalol has been used for several decades as the first choice in the treatment of hypertension. It combines good pharmacological activity and low secondary adverse effects due to its effects on the alpha- and beta-adrenoreceptors.[1,2] Acute crises are usually treated with intravenous administration of the drug, but for chronic control of the arterial pressure immediate-release solid oral dosage forms are employed[3] despite its variable oral bioavailability.[4,5]

Oral bioavailability of drugs depends on numerous factors affecting intestinal absorption, intestinal secretion, and intestinal and hepatic metabolism that can lead to nonlinear dependency on concentration. Nonlinear kinetic phenomena are very often responsible for variability. Therefore, an understanding of the mechanisms causing such nonlinear behavior would be helpful in predicting the influence of dosage, the possible drug–drug and drug–food interactions and in optimizing the clinical use of drugs.[6]

The aim of this work was to study the labetalol absorption process, as the first step that can influence its bioavailability. In order to differentiate absorption from intestinal metabolism, the *in situ* intestinal perfusion technique was used as it is based on the disappearance of the drug in the luminal fluid. A wide range of concentrations was assayed to test the linearity of the process. As the absorption constant depends on the concentration, the hypothesis of a nonlinear mechanism of absorption is consistent. The kinetic analysis of the data was performed by a simultaneous fit of the concentration versus time data sets to a group of

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differential equations representing different biophysical models of absorption. Mathematical evaluation of the fit allows the selection of the most probable model as an evidence of the actual mechanism. The possible involvement of carrier-mediated processes was also checked by inhibition studies.

MATERIALS AND METHODS

Absorption Tests

The animal experimentation was approved by the Pharmacy Faculty Ethics Commission. *In situ* rat gut preparation,[7] modified as previously reported,[8,9] was used for absorption test. Animals were obtained from the authorized Faculty facility, where the housing conditions are a 12 h day/night cycle and 21°C. Male Wistar rats weighing 250–300 g fasted for 20 h but with free access to water were anesthetized 1 h before surgery by 2% (w/v) intraperitoneal injection of pentobarbital 40 mg/kg of animal weight.

Experimental data were obtained from two regions of the rat intestine: small intestine and colon.

Initially, perfusion assays were performed in the whole length of the small intestine, where the biliary duct was previously ligated. After rinsing with physiological saline solution (25 mL) in order to eliminate fecal residues and debris, 10 mL of one of eight labetalol solutions (2, 7.5, 10, 50, 100, 500, 1000, or 2000 μM), prepared in saline buffered to pH 6.4 (10% v/v phosphate buffer 0.066 M), was perfused at 37°C ($n = 6$). Inhibition studies were then carried out. In the first series of inhibition experiments, 10 mL of one of two labetalol solutions (10 or 100 μM) plus verapamil 7 mM was perfused in the whole small intestine ($n = 6$). For the second series of inhibition assays, 10 mL of one of two labetalol solutions (10 or 100 μM) with grapefruit juice diluted with bidistilled water (50/50, v/v) was perfused as above ($n = 6$). In a third series of inhibition assays, one solution of labetalol 10 μM with p-aminohippuric acid 10 mM was also assayed.

Several perfusion assays were performed in the whole colon according to a similar protocol as described above. In this case, a volume of 5 mL of 2, 50, 100, 500, or 1000 μM labetalol solutions, buffered to pH 6.7, was perfused at 37°C ($n = 6$) in the prior experiments. For the following series, 5 mL of a 100 μM labetalol solution in the presence of verapamil 7mM or grapefruit juice diluted with bidistilled water (50/50, v/v) was made isotonic and perfused ($n = 6$).

In all cases, the remaining concentrations of the drug in the intestinal lumen were measured every 5 min, for a total time of 30 min, taking 300 μL samples of the perfused solutions. The stability of labetalol in the intestinal media was checked to ensure that the losses were due to the absorption process.

Water reabsorption during the experiment was evaluated and corrected separately for each animal using a direct volumetric procedure already described.[9,10]

The above-mentioned *in situ* technique was also used to evaluate the effect of the verapamil on the intestinal permeability. For this purpose antipyrine was selected, since it is known to be only passively absorbed.[11] Therefore, solutions containing antipyrine 133 μM with and without verapamil 7 mM were perfused in the small intestine of the rat.

Analytical Procedures

Intestinal samples were assayed for labetalol content by HPLC. The equipment used was a Hewlett-Packard (HP Barcelona, Spain) 1050 pump, an HP 1046A fluorescence detector with excitation and emission wavelengths of 331 and 419 nm, respectively, and an HP 3395 integrator.

The stationary phase was a Novapak (150 × 4.6 mm) column (Waters, Barcelona, Spain), fitted with a C-130B precolumn Teknokroma C-18 (Teknokroma S.L., Barcelona, Spain). The mobile phase was 25% (v/v) acetonitrile (Scharlab, Barcelona, Spain) and 75% (v/v) of 0.05 mM solution of orthophosphoric acid (Scharlab) (pH 7.2) with a flow of 1.0 mL/min through the column.

Samples were centrifuged at 3000 rpm for 10 min, and 30 μL of the supernatant was injected onto the column using an Agilent 1100 Series autosampler (Agilent Technologies, Barcelona, Spain).

Calibration curves in the range 0.5–2000 μM were prepared. Linear plots relating the peak areas and the labetalol concentrations were obtained. The accuracy and precision of the method were validated. Both criteria were assessed using different labetalol concentrations in the absence and in the presence of verapamil, p-aminohippuric acid, or diluted grapefruit juice, covering the entire calibration range of the