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INTESTINAL BIOAVAILABILITY OF ACEBUTOLOL (ANTI-HYPERTENSIVE AGENT) AFTER PRETREATMENT OF DEXAMETHASONE (IM) IN RATS: EVIDENCE OF P-GLYCOPROTEIN INDUCTION

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ABSTRACT

For investigation, the effect of P-glycoprotein (P-gp) inducer agent as Dexamethasone HCL (DEX) on P-gp expression gene using of the anti-hypertensive agent Acebutolol HCL (ALH), in situ rat Single -Pass Intestinal Perfusion (SPIP) technique was applied. Rats were divided into two groups. The first group was perfused with ALH only (260 µg/ml). The second groups were treatment with DEX (2.4mg/kg) intra-muscular injection daily along 5 days before applied ALH as first group. The samples of the perfused solutions were collected in certain intervals. The analysis was performed using a simple, rapid and validated spectroscopic method. The results demonstrated that the mean absorption rate constant (Ka) in first, and second groups was 0.46 ± 0.092 hr-1 and 0.21 ± 0.03hr-1 respectively. The decreased value of Ka (2.3 folds) upon treatment with DEX. The results indicated that P-glycoprotein regulated with P-gp inducer agent as DEX that lead to decreasing the intestinal absorption of ALH, which can explain its lower bioavailability after oral administration. It can explain also the possible drug-drug interactions of ALH with P-gp inducer until it's not co-administration at same day.

KEY WORDS

P-glycoprotein, Acebutalol, Single-Pass Intestinal Perfusion, Dexamethasone.

INTRODUCTION

Acebutolol, N- [3-Acetyl–4- [2–hydroxy–3- [(1 methyl ethyl) amino] propoxy]-butanamide, is selective β_1-Adrenoceptor antagonists, but less active in blocking β_2-adrenoceptors of the autonomic nervous system figure 1 (Brunton, Parker and Lazo, 2006).

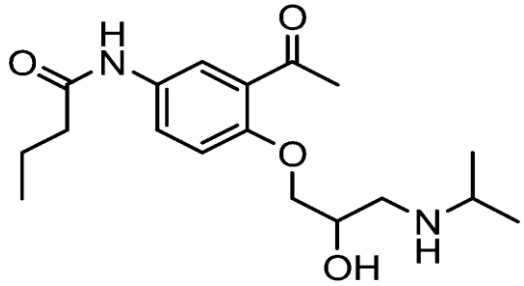

Figure 1: Chemical structure of Acebutolol

ALH is complete absorbed after oral administration, because of the first-pass-metabolization, leading to a bioavailability 35% to 50% only. The plasma peak concentration was reached within 2 to 2.5 hours and a half-life of 3 to 4 hours (Reynolds, Martindale, 1996).

Bioavailability of drugs after oral administration affected by many factors that affecting on intestinal absorption such as intestinal secretion, and intestinal metabolism (Burton et.al, 2002). P-glycoprotein (P-gp) consider the main intestinal secretion mediator which distributed along Gastrointestinal tract (GIT), that pumped the drugs absorbed out to intestinal luminal fluid which lead to decreased the amount reach to blood phase (Pang, 2003, Lin and Yamazaki, 2003).

DEX consider P-gp inducer and increasing of P-gp mRNAs expression gene, that lead to increasing of P-gp present and function along GIT which responsible of non-linear pharmacokinetic of P-gp substrate drugs (Fardel, Lecureur and Guillouzo, 1993).

The aim of the present study was to study ALH absorption process and to investigate the effect of P-gp inducer agent (DEX) on P-gp gene expression and function along of time, using in-situ SPIP though the whole rat intestine.

MATERIALS AND METHODS

Materials

Acebutolol hydrochloride and Dexamethasone hydrochloride standards were purchased from Sigma-Aldrich (Germany). Normal saline (0.9% w/v) was obtained from pharmaceutical solution industry (kingdom of Saudi Arabia) and Thiopental sodium (500 mg vial) was obtained from Egyptian INT. Pharmaceutical Industry (Egypt).

Instrument

SHIMADZU (UV-1601) Spectrophotometer and 1 cm quartz cells were used. Centrifugation was made with Kokusan (H-103N) Series Centrifuge. A hot plate (P/Selecta) was required.

Animals

Twelve adult Wistar albino male rats (weighted: 250-300 g, aged: 7-9 weeks) were obtained from Cairo University (Cairo, Egypt). Animals were housed two per polypropylene cage, at relative humidity of 60 %. An approval for study conduction was obtained from Helsinki Committee (Gaza, Palestine). All experiments with rats were conducted according to the C1anadian guide for the care and use of laboratory animals (Dawson et. al., 1986).

Method Development

Preparation of standard stock solution

Standard solution of ALH was prepared by transferring accurately weighed 13mg of drug into a 50ml volumetric flask with normal saline to get the concentration of 260µg/ml.

Preparation of calibration curve

From the standard stock solution, fresh aliquots were pipette out and suitably diluted with normal saline to get final concentration in the range of 5-50 µg/ml. The solutions were scanned under 200-400 nm wave length range and a sharp peak was obtained at 234nm (figure 2). The calibration curves were obtained by the analysis of standard samples containing ALH at the concentration of 5, 10, 18, 24, 30, 36, 45 and 50 µg/ml. Finally, calibration curve was plotted by taking absorbance on y-axis and concentration of solution on x-axis. Regression analysis was performed to describe the linear curve obtained (figure3).

Preparation of perfusion solution:

DEX (0.72mg/ml): 0.72 ml DEX ampoule (4mg/ml) dissolved in 1 ml normal saline, take 0.25ml of solution to injection the rats Intramuscular injection (0.72mg/0.25ml).

In situ SPIP

Preparation of animals:

In situ rat model, using small intestine was following the traditional Sing-Pass Intestinal Perfusion (SPIP) technique (Doluisio et. al., 1969). Exposed the small intestine by a midline abdominal incision, and inserted two L-shaped glass cannulae through small slits at the duodenal and ileal ends. Then, Using silk suture to legated biliary duct. Taken the care when handle the small intestine gently and to maintain an intact blood supply. The cannulae were secured by ligation with silk suture, and the intestine was returned to the abdominal cavity to aid in maintaining its viability and integrity, then using cotton pad soaked with warm normal saline (37°C) to covered small intestine to prevent dryness of intestine and maintaining the body temperature. 4-5-centimeter segments attachment of Tygon tube to the exposed ends of both cannulae, and a 30-ml hypodermic syringe fitted with a three-way stopcock which containing perfusion fluid warmed to 37°C was attached to the duodenal cannula. As an aim of clearing the intestine, fluids were perfusion slowly through gut discarded until the effluent clear solution. The remaining fluids was expelled carefully from the intestine through using of air pumped syringe, finally, 10 ml of drug solution was immediately pumped through fixed three-way stopcock into the intestine by using the syringe. The stopwatch was adjustment, and the ileal cannula was connected to another syringe fitted with a three-way stopcock. This arrangement enabled the operator to sample the intestinal lumen solution into either the ileal or the duodenal side, remove a 200 µl aliquot from intestinal luminal fluid within 10-15 sec. To assure homogeneity drug solution concentrations along the intestinal segment, samples were taken from the two syringes alternately. When the experiment was ended, the rat was euthanatized with a cardiac injection of saturated solution of KCl.

Absorption studies of ALH:

A 10 ml of ALH solution at a concentration (260 µg/ml) was perfused into small intestine segment of five rats (the first group). The second rat groups were treatment with DEX IM (0.72mg/0.25ml) daily along 5 days before SPIP experimental, then perfused with 10 ml solution containing ALH (260 µg/ml). 200 µl of luminal intestinal fluid samples were collected from duodenal and ileal sides alternately at different time intervals 5, 10, 15, 20, 25, and 30 min. Samples were diluted to 3 ml with normal saline and centrifuged at 5000 rpm for 5 min. The supernatant was separated then filtered through whatmann filter paper and kept at room temperature until being analyzed. Absorption was measured at 234 nm against blank (Moffat and Clarke's, 1986). The amount of acebutolol was computed by using the equation referring to the calibration curve (figure 3).

Data analysis

Data analysis was performed by Statistical Package of Social Sciences SPSS version 13 (SPSS, 1997).

RESULTS AND DISCUSSION

UV Spectrum

As shown in Figure 2, the peak of ALH in normal saline exhibited a maximum absorbance at 234nm.

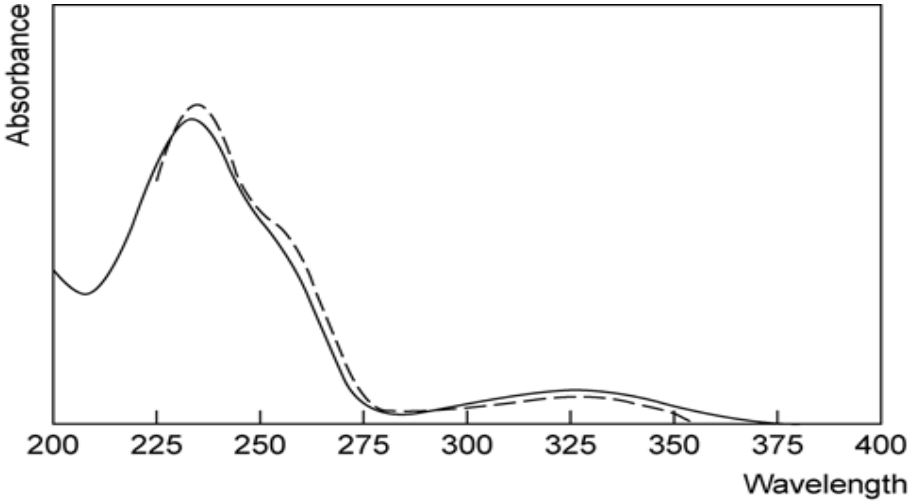

Figure 2: UV spectrum of acebutolol in normal saline

Determination of ALH by Ultraviolet Spectrophotometry

The linear regression equation and the standard curve which was used for the assay of ALH is presented in Figure 3.

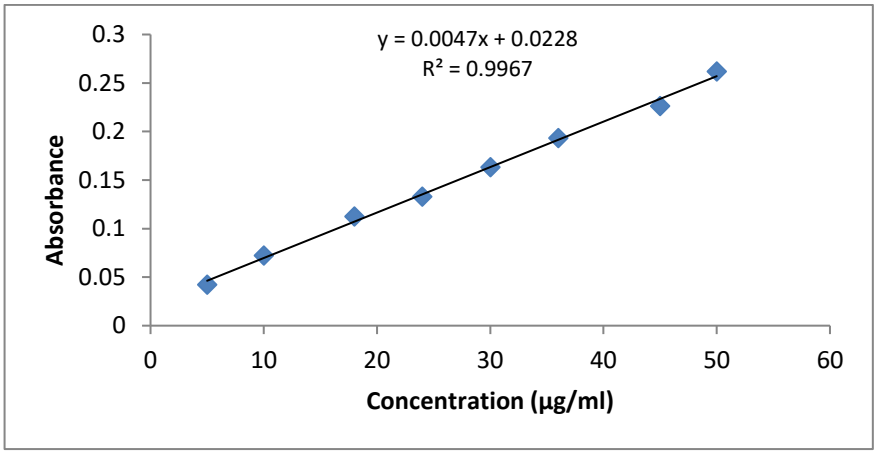

Figure 3: Spectrophotometric calibration curve of ALH

Validation of the analytical method

The analysis was performed by direct spectrophotometric assay of collected intestinal fluid samples. The selected wavelength was at 234 nm, where there were no interferences from intestinal components or VLH recorded. The method was validated for different parameters like linearity, accuracy, precision and stability (Abushammala I. and El-Qedra A., 2013*).*

Linearity

Fresh aliquots were prepared from the stock solution (26µg/ml) in different concentrations. The samples were scanned in UV–visible spectrophotometer against reagent blank. It was found that the selected drug shows linearity between the 5-50 µg/ml (Table 1).

Table 1: Linearity results of Acebutolol in normal saline

Concentration (µg/ml)	Absorbance
5	0.0423
10	0.072
18	0.1123
24	0.1326
30	0.1633
36	0.1933
45	0.2263
50	0.2617
R^2	0.9967
Intercept	0.0228
Slope	0.0047

Accuracy

Six different concentration of ALH in range of 5, 10, 18, 24, 30, and 36 µg/ml were analyzed and the ALH content of each sample was calculated. Percent recovery was determined by comparing the true content with that of calculated. Accuracy of the analytical method was confirmed by means of statistical evaluation.

Precision

Precision (intra-day precision) of the method was evaluated by carrying out the six independent test samples of the acebutolol. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts, and different days in the same laboratory. The percent relative standard deviation (%RSD) obtained was found to be good. The results were tabulated in Tables (2&3).

Table 2: Intra-day Precision (System Precision)

Concentration (µg/ml)	Mean	%SD	%VC
5	5.065	0.1380	2.724
18	18.061	0.1260	0.6976
50	49.913	0.5521	1.106

Table 3: Inter-day Precision (Method Precision)

Concentration (µg/ml)	Mean	%SD	%VC
5	5.13	0.1442	2.810
18	18.271	0.1593	0.872
50	50.228	0.2474	0.492

SD: Standard deviation, VC: Variation Coefficient

Stability

A stability test was made to be sure that the ALH remained stable during the course of the analysis. In order to study the stability of ALH under test condition (at room temperature), the spiked samples of drug at the concentration of 5 and 18 µg/ml were measured freshly and after 8 hours Table 4.

Table 4: Stability of ALH during the analytical procedure

Time (h)	Concentration (µg/ml)	Concentration obtained (µg/ml)	CV%	Recovery%
0	5	5.11 ± 0.146	2.85	102.2
8	5	5.22 ± 0.103	1.97	104.4
0	18	18.18 ± 0.124	0.68	101
8	18	18.34 ±0.09	0.49	101.8

Absorption studies of ALH

In the present study, ALH was perfused into the whole rat intestine by SPIP technique. The gradual decrease of remnant ALH concentrations with time, indicated that ALH absorption followed first-order kinetic (Figure 4).

The absorption rate constant was calculated according to the following equation:

$$\ln Ct = \ln Co - Ka.t$$

1- **Ct**: Concentration of drug after t time of perfusion
2- **Co**: Initial drug concentration,
3- **ka**: Absorption rate constant and
4- **t**: Time. The mean absorption rate constants ka was 0.46 ± 0.092 hr-1and 0.21 ± 0.03hr-1 for the first and second groups, respectively (Table 5). The absorption rate constant was decreased by 2.3 folds upon pervious treatment with DEX, a P-gp inducer (Figure 5).

Table 5: Calculated parameter of ALH

	First group	Second group
Ka (hr^{-1})	0.46 ± 0.092	0.21 ± 0.03
%A°	98.9 ± 0.20	99.7 ± 0. 12
R	0.97 ± 0. 011	0.99 ± 0.023

***ka**: Absorption rate constant, % **Ao**: Estimated inclination of the rectal absorption line, R: Correlation coefficient, Rat **groups**: first group perfused with ALH (260 µg/ml), second groups perfused with ALH after treatment with DEX (2.4mg/kg) I.M injection daily along 5 day.*

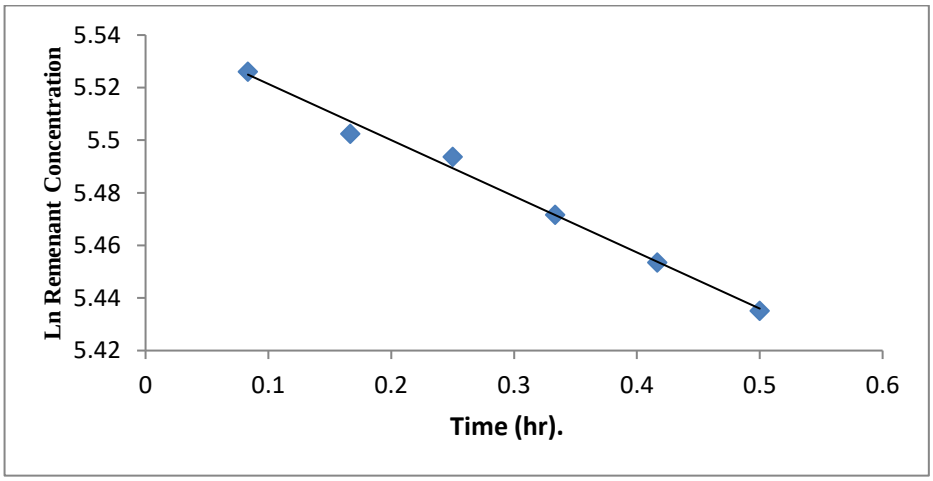

Figure 4*: Graphical representation Plot of the fit of the apparent First-order equation to the mean data (remaining luminal concentrations of 260 µg/ml ALH).*

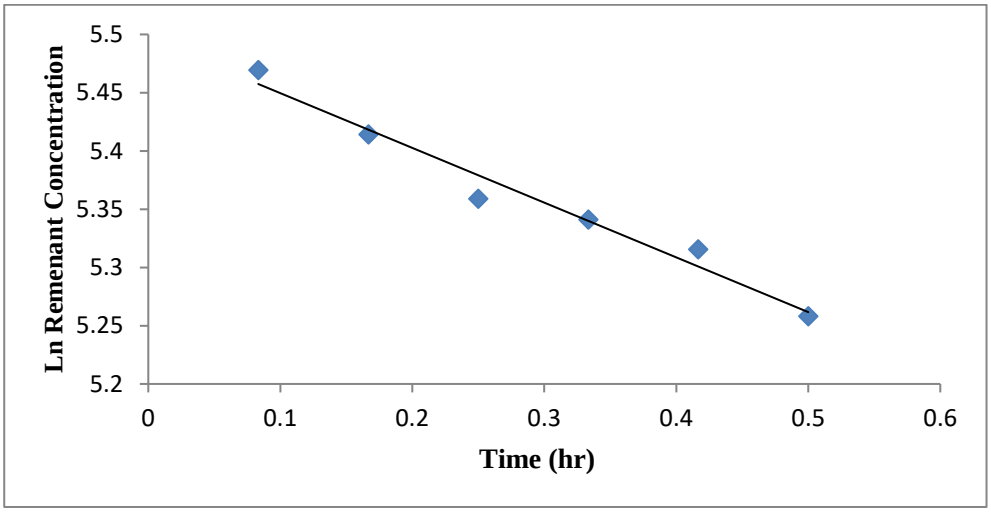

Figure 5*:* Graphical representation Plot of the fit of the apparent First-order equation to the mean data (remaining luminal concentrations of 260 µg/ml ALH after treatment with DEX 2.4 mg/kg).

Statistical analysis of data

One way ANOVA test:

The first statistical test was performed is one way ANOVA test to check the homogeneity within a group (Table 6). A minimum inter-individual variation among rats per group was estimated (p-value > 0.05). It was noted that, the intestinal permeability rate within rats aged 5-30 weeks showed low differences (Lindahl et. al., 1997).

Table 6: One way ANOVA test

Rat group	N	F-value	P-value
First group	6	1.23	0.382
Second group	6	0.834	0.592

Rat groups: *first group perfused with ALH (260µg/ml), second groups perfused with ALH in after treatment with IM injection of DEX(2.4mg/kg) daily, along 5day.*

Bonferroni Test

The constant rate of ALH absorption in free solution was a critical step for studying the effect of DEX on P-gp distributed and functional, ALH absorption phase and to be compared with those obtained when a specific P-gp inducer DEX was treatment previously. DEX was choice as a specific efflux P-gp inducer. Bonferroni test (table 6) noted that there was a statistically significant difference between two groups, when ALH was perfused alone (first group) and when perfused with ALH in after treatment with IM injection of DEX daily, along 5day. These results confirmed that, ALH is a substrate for rat intestinal P-gp, and a crucial role of P-gp was played in the uptake of ALH from the intestine. A treatment DEX along 5day crucial role of P-gp distributed and functional increased that showed statistically significant difference on two group (p- value < 0.05).

Table 6: A multiple comparison Bonferroni test of rat groups.

Group	Group	Standard error	P-value
First group	**Second group**	0.0054	0.000*

**Statistically significant (p-value < 0.05), rat groups: first group perfused with ALH (260 µg/ml), second groups perfused with ALH in after treatment with IM injection of DEX(2.4mg/kg) daily, along 5day.*

A similar study the effect of P-gp inducer (DEX) on vincristine across Graphene-Nano-technique (GPNT) monolayers was close to the permeability coefficient of bovine brain endothelial cells co-cultured with astrocytes, that noted DEX resulted in decreased uptake of vincristine (P-gp substrate) without any increase in P-gp expression (Regina et. al. 1999).

Another study, that examined P-glycoprotein (P-gp) expression and function in cultured rat hepatocytes in response to dexamethasone (DEX), the results indicating that high-expressed P-gp functional. These results explain that DEX treatment strongly inducer P-gp expression in primary rat hepatocyte cultures through a specific effect on the mdr1 gene (Fardel, Lecureur and Guillouzo, 1993).

A recent study of expression and activity of p-glycoprotein level by dexamethasone in cultured retinal pigment epithelium included glucocorticoid receptor, a significant increasing on mRNA and protein levels as well as by functional activity of P-gp was founded, were induced within 12 hours of dexamethasone treatment, persisted as long as 24 hours (Zhang et. al. 2012).

CONCLUSION

P-gp are responsible for low bioavailability of ALH, in other side, P-gp gene expression are increasing when long exposure to P-gp inducer agent as DEX that lead to increasing the number of P-gp distributed along GIT that effect on pharmacokinetics especially absorption phase of all P-gp substrate drugs.

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