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EFFECT OF REPAGLINIDE, A P-GLYCOPROTEIN MODULATOR ON ABSORBTION OF PHENYTOIN

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ABSTRACT

P-glycoprotein, an efflux transporter prevents intracellular accumulation of toxins and xenobiotics. The present research work aims to investigate the effect of repaglinide on absorption of phenytoin due to the evidence of repaglinide having affinity for P-glycoprotein. The objective of the study was to assess apparent permeability coefficient of phenytoin in the presence of repaglinide by non-everted sac technique. The results of ex-vivo study showed that phenytoin level decreased in a time and dose dependent manner ascertaining the inducing role of repaglinide on P-glycoprotein leading to the decrease in concentration of phenytoin which may lead to therapeutic failure. Hence this combination may require close monitoring for better therapeutic outcome.

KEYWORDS: P-glycoprotein, an efflux transporter prevents intracellular accumulation of toxins and xenobiotics.

INTRODUCTION

P-glycoprotein (P-gp) is an active, efflux; expressed in the apical membranes of intestine, live kidney and capillary endothelial cells of the blood brain-barrier.[1, 2] P-gp due to genetic polymorphism may affect drug therapy in two ways first, an increase in expression of P-gp may reduce drug bioavailability leading to therapeutic failure and secondly it may decrease P-gp expression and thereby produces drug toxicity.[3] Also it is highly expressed in cancer cells, leading to efflux of anticancer agents from cells leading to chemotherapeutic drug resistance.[4]

Phenytoin an anti-epileptic drug is most effective in the generalized tonic-clonic and patrial seizure. Repaglinide is an oral blood glucose lowering drug of the meglitinide class used in the management of type 2 diabetes mellitus. It lowers blood glucose levels by stimulating the release of insulin from pancreas. Phenytoin is the substrate of P-gp and in-silico and in vitro cell culture studies show that repaglinide has P-gp affinity.[5] Thus we hypothesize possible drug interaction between these two drugs mediated by P-gp. The aim of the present study was to investigate effect of repaglinide, a P-gp modulator on absorption of phenytoin.

MATERIALS AND METHOD

Phenytoin (Panacea Biotec Ltd., India) and repaglinide (A-TRET, Kaizen Research Labs India Pvt Ltd.), LDH and Glucose kit (Randox Laboratories Pvt Ltd., India) were purchased and double beam UV spectrophotometer (Thernoficher Pvt Ltd., USA) was used.

Non –everted sac technique

Chicken intestine was procured from slaughter house.[6] The small intestine was removed and flushed with 50ml ice-cold saline. The jejunum was isolated, and a segment was cut into length of 10 cm for preparation of the sac. Drug treatment consists of three different groups. Group 1- Phenytoin (50µM), Group 2- Phenytoin (50µM) + Repaglinide (200µM), Group 3- Phenytoin (50 µM) + Repaglinide (400µM). Tyrode's solution containing the respective combinations was introduced into the non-everted sac (mucosal side), and both ends of the sac were ligated tightly. The sac was mounted in 40ml organ bath with Tyrode solution oxygenated with 5% CO2 / 95% O_2 at 37^o c. Drug sampling has been done at 30, 60, 90, 120 mins from the organ bath and replenished with fresh buffer. Phenytoin was determined using UV spectroscopy at 204nm. The LDH and glucose level of non - everted sac was measured at 30, 60, 90 and 120 mins. The apparent permeability coefficient for phenytoin was calculated from the equation given below.

$$P_{app} = \frac{dq}{dt} \cdot \frac{1}{ACO}$$

Where, dQ/dt- The rate of drug transport from mucosal to serosal medium

A- The surface area of the intestinal sac used for the study (cm^2)

CO- The initial concentration of drug present in the intestinal sac.

Lactate dehydrogenase (LDH) release in the non-everted sac

The viability and any possible damage of the gut were evaluated by measuring the release of the cytosolic enzyme lactic dehydrogenase (LDH) as an indicator of cell damage. The LDH of non everted sac was measured at 30, 60, 90 and 120min compared to the phenytoin treated group.[7] The LDH activity was determined by using LDH kit from Randox, using autoanalyser.

Glucose transport across the non-everted sac

The viability and the integrity of the gut sacs were further demonstrated by analyzing the glucose concentrations both in the mucosal and serosal sides. Samples of incubation medium and content of the sacs were collected at predetermined times and glucose concentrations were measured by kit from Randox using autoanalyser.

RESULTS

Table 1: Apparent permeability coefficient (P_{app}) for phenytoin in presence or absence of repaglinide using non- everted sac technique.

Drug treatment	Apparent permeability(P_{app}) Cm/s			
	30mins	60mins	90mins	120mins
Phenytoin(50µM)	0.394	0.429	0.432	0.432
Phenytoin(50M) + Repaglinide (200µM)	0.0462	0.0462	0.0466	0.0469
Phenytoin(50µM) + Repaglinide (400µM)	0.0848	0.0852	0.0860	0.0876

Data expressed as mean (n=2).

From table 1, the apparent permeability coefficient (P_{app}) of phenytoin decreased in time and dose dependent manner compared to phenytoin alone treated group.

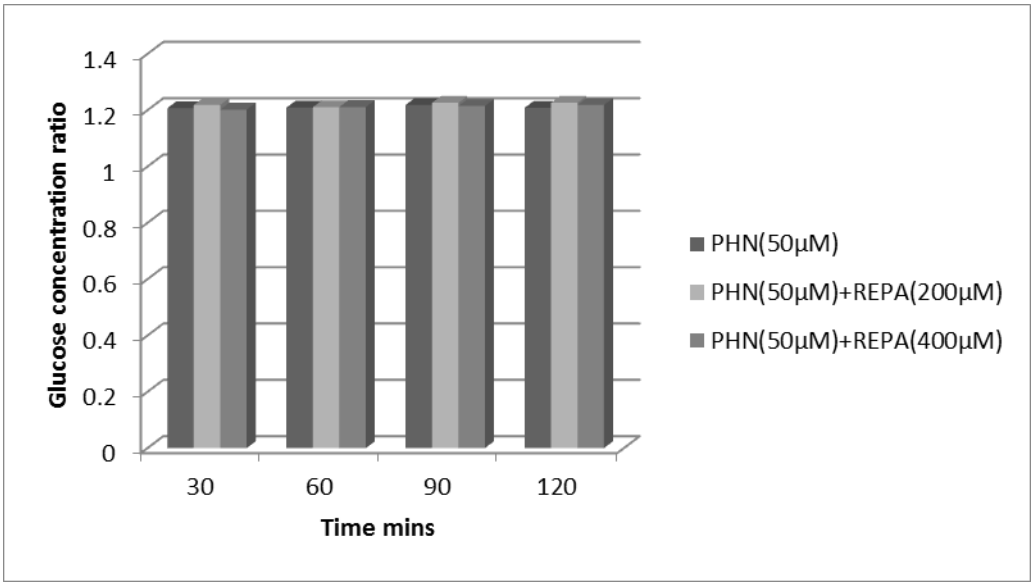

Figure 1: Glucose transport in non-everted sac model.

Data are shown as mean (n =2).

PHN- phenytoin, REPA- repaglinide

As shown in Figure 1 in phenytoin (50µM), phenytoin (50µM) with repaglinide (200µM), and phenytoin (50µM) with repaglinide (400µM) treated groups glucose concentration inside the sacs (mucosal side) was approximately 1.2 times higher than the outside (serosal) concentration, indicating that the tissue of gut sac was viable and well-functioning.

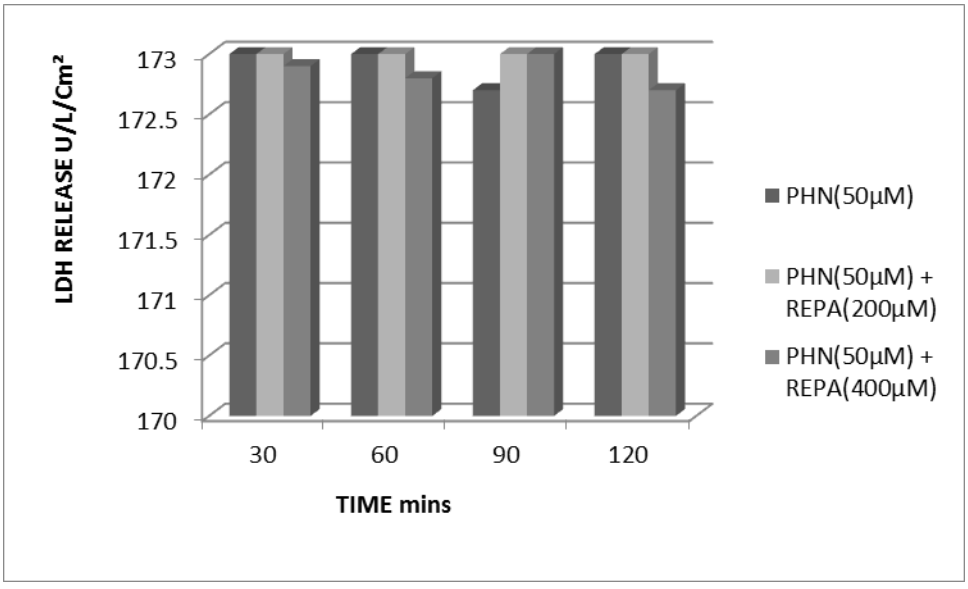

Figure 2: LDH release in non-everted sac model.

Data are shown as mean (n = 2).

PHN- phenytoin, REPA- repaglinide

The LDH of non everted sac was measured at 30, 60, 90 and 120 min (Fig. 2). The results reveal that there was no significant difference in LDH level till 120min implying the integrity of the tissue was maintained till 120 minutes.

DISSCUSSION

Result of *ex vivo,* study showed that phenytoin level decreased in a dose and time dependent manner with co-administration of repaglinide. This decrease in level of phenytoin may be due to induction of P-gp by repaglinide. Induction of p-glycoprotein will reduce the bioavailability of phenytoin which may lead to therapeutic failure. However further *in vivo* studies are required to throw light in this regard.

CONCLUSION

Concentration of phenytoin decreases when combined with repaglinide by inducing P-gp transporter. Hence, it shows that repaglinide may act as P-gp inducer.

REFERENCES

1. Sharom FJ, The P-Glycoprotein Multidrug Transporter, Essays Biochem, 2011; 50(1): 161-78.
2. Saaby L, Brodin BA, Critical View on In vitro Analysis of Pglycoprotein (P-gp) Transport Kinetics, Journal of Pharmaceutical Science, 2017; 106(9): 2257-64.
3. Linardi LR, Natalini CC. Multi-drug resistance (MDR1) gene and P-glycoprotein influence on pharmacokinetic and pharmacodymanic of therapeutic drugs. Ciência Rural, Santa Maria, 2006; 36(1): 336-7.
4. Shikawa JT, Hirano H, Onishi Y, Sakurai A, et al., Functional Evaluation of ABCB 1 (P-Glycoprotein) Polymorphisms: HighSpeed Screening and Structure-Activity Relationship Analyses, Drug Metab Pharmacokinet, 2004; 19(1): 1-14.
5. Chang C, Bahadduri PM, Polli JE, Swaan PW, et al., Rapid identification of P-glycoprotein substrate and inhibitors. Drug metabolism and disposition, 2006; 34(12): 1976-84.
6. Madar Z, Demonstration of amino acid and glucose transport in chick small intestine everted sac as a student laboratory exercise. Biochem Educ, 1983; 11: 9–11.
7. Parsa A, Saadati R, Abbasian Z, Azad Aramaki S, et al., enhanced permeability of etoposide across everted sacs of rat small intestine by vitamin E-TPGS, Iran J Pharm Res, 2013; 12: 37-46.